CHEMICAL AND PHYSICAL PROPERTIES OF BREAKFAST CEREALS AND SNACKS MADE FROM SPECIALTY SORGHUMS AND SORGHUM BRAN USING TWIN SCREW EXTRUDER

A Dissertation

by

MUHAMMAD ASIF

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2011

Major Subject: Food Science and Technology

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ABSTRACT

Chemical and Physical Properties of Breakfast Cereals and Snacks Made from Specialty Sorghums and Sorghum Bran Using Twin Screw Extruder.

(December 2011)

Muhammad Asif, B.Sc.; M.Sc., University of Agriculture, Faisalabad

Co-Chairs of Advisory Committee: Dr. Lloyd W. Rooney
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Whole ground white, high tannin and black sorghum with and without additional high tannin sorghum bran were used in different proportions to develop ready to eat breakfast cereals and snacks. The effect of extrusion on the phenolic compounds and on *in vitro* starch digestibility of sorghum based cereals and snacks were observed.

Gluten free and gluten containing breakfast cereal and snacks were developed with different physical, chemical and sensory characteristic. By increasing the sorghum and bran level in the formulations, the bulk density of extrudates was increased while expansion ratio was decreased. Bowl life of extrudates was increased up to 18 min. when 60% whole ground white sorghum was used with additional 10% high tannin sorghum bran.

Water soluble index was significantly higher for the extrudates without additional bran and decreased as bran was added. A positive correlation between water soluble index and expansion ratio (R^2=0.89) indicated that the more expansion ratio

provided a large surface area for water to interact with starch and other soluble components.

The retention of total phenols in these extrudates varied from 13-41% and it was found that extrudates with additional high tannin sorghum bran had more total phenols than extrudates without it. Sorghum extrudates showed a significant reduction in antioxidant activity varied from 21-83%. Similarly, the effect of extrusion on condensed tannins was detrimental, and their retention was ranged from 12-28%. The smaller particle size of ground sorghum increased the surface area of contact between composite flour components and extruder barrel which promoted interactions during extrusion, lowering condensed tannins and antioxidant activity.

All sorghum based extrudates had significantly ($P<0.05$) lower starch digestibility than that of corn flour extrudates. All types of sorghum had non-significant difference in starch digestibility from 0.5-2hrs. After 16 hrs., high tannin sorghum extrudates had the lowest starch digestibility (79%), which was significantly different from other sorghum types. There was a negative correlation between the rapid digestible starch and tannin contents (R^2=0.62). Breakfast cereals made from different types of sorghum and bran levels were statistically equally rated in taste and overall acceptability.

DEDICATION

I dedicate this manuscript to

My advisor “Dr. Lloyd W. Rooney” and my father “Abdul G. Tahir”

ACKNOWLEDGEMENTS

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and management which were lacking in my career. I am very thankful to Rick Flaget and Edward Lawes for guiding me in my weaknesses and showing me the ways to strengthen my strengths. I cannot forget the care and cooperation of Robert Stackow and his wife Veronica Egan, both are not only experts in their areas but are always very kind with me. I am proud for having opportunities to work with Jim Kincaid, Bin Fu, Viswas Ghorpade, Badri Narayanan, Jerry Rutherford, Lisa Unlu, Tim VandeGiessen, Sara Williams, Marc Barron, Shawn Busse, Monjur Hossen and many others. Special thanks are due to Fred Linabury for guiding me in some important aspects of R&D. I am also very thankful to Cherie Floyd for taking care and supporting me through my transition period in Kellogg. I am very obliged to John Kepplinger for being optimistic about me.

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NOMENCLATURE

BD	Bulk Density
CD	Celiac Disease
EGI	Estimated Glycemic Index
ER	Expansion Ratio
GI	Glycemic Index
HDL	High Density Lipoprotein
HI	Hardness Index
HPLC	High Performance Liquid Chromatography
LDL	Low Density Lipoprotein
RDS	Rapid Digestible Starch
RS	Resistant Starch
RTE	Ready-to-Eat
SDS	Slow Digestible Starch
SKHT	Single Kernel Hardness Tester
SME	Specific Mechanical Energy
TKW	Thousand Kernel Weight
WAI	Water Absorption Index
WSI	Water Soluble Index

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1. INTRODUCTION: THE RESEARCH OBJECTIVES

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the five top cereal crops in the world, along with wheat, oats, corn and barley (FAO, 2007) and is a staple food for many in the arid, dry climates of Africa and Asia. Sorghum grains are round, made up of pericarp, endosperm, and germ. It is a unique cereal grain with starch granules present in the pericarp (Rooney & Serna-Saldivar, 2000).

Sorghum grain has been described as a good source of nutrients, phenolic compounds, neutral in flavor and sometimes slightly sweet therefore it makes very adaptable flour for multiple uses. Sorghums can be milled directly into whole-grain flour to produce cookies, cakes, brownies, breads, pizza, pasta, pancakes, waffles, fermented and unfermented flat bread, porridges, malted into alcoholic and non-alcoholic beverages and extruded products.

Significant increased interest in sorghum for special food production has encouraged production of better quality sorghum flours. These flours vary in color depending upon the type of sorghum and processing capability. Usually sorghum is decorticated to remove the bran and part of the germ. This operation is done with a rice pearler or other abrasive mill. Some special sorghums like black, tannin, yellow and others have high levels of phytochemicals that are unique in terms of their healthy components which include high levels of rare 3-deoxy anthocyanins, condensed tannins and various other

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This dissertation follows the style of *Food Chemistry.*

combinations (Dykes & Rooney, 2006; Shahidi & Naczk, 2004). Sorghum phytochemicals inhibit tumor development and show high antioxidant activity against different free radicals *in vitro* and have benefits similar to fruits and vegetables (Awika & Rooney, 2004).

Tannin and black sorghums are high in phytochemicals (Awika, 2003), they have a potential impact against obesity, cancer and type II diabetes. These varieties are less digestible than most other cereals (Rooney & Pflugfelder, 1986). Tannin sorghum extrudates and porridges have reduced glycemic index (EGI) and increased resistant starch values compared to corn extrudates and porridges (Palomino-Siller, 2006). Tannin sorghum, a good source of antioxidant activity could be used to reduce the digestibility of food in diabetic patients and control weight in obese persons. Sorghum bran is a good source of dietary fiber (Rooney et al., 1992), which aids in gastrointestinal health through bulking fecal matter, decreasing constipation, and reducing the absorption of carcinogenic metabolites (Kahlon et al., 2001).

Consumer demand for gluten-free products is rising steadily in the US with the increase in Celiac disease (CD) and other allergic reactions to gluten. CD is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. The only treatment of this disease is a gluten free diet. CD is an inherited autoimmune disease that affects the small intestine (Gallagher, 2009). When a person with CD consumes gluten, the individual's immune system responds by attacking the small intestine and inhibiting the absorption of important nutrients. Therefore people with CD must adhere to a gluten-free diet. This diet involves strictly avoiding wheat, barley and rye. Pure oats are okay but they may be contaminated with wheat, barley or rye. Sorghum's popularity as a gluten free cereal continues to grow due to its bland flavor, low cost, increasing availability, and its ability to be blended into various products without eclipsing the tastes of the other ingredients.

Preliminary experiments were conducted to develop breakfast cereals and snacks by using 50-80% whole ground sorghum with additional 0-10% high tannin bran. Other ingredients used were yellow corn flour, corn starch and rice flour. In these experiments whole ground sorghum was used to fulfill the requirements of whole grain products. According to American Association of Cereal Chemist, the current definition of whole grain foods and ingredients "whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components, the starchy endosperm, germ and bran, are present in the same relative proportions as they exist in the intact caryopsis" (AACC, 1999).

The whole ground sorghum and high tannin bran increased the water soluble index (WSI), water absorption index (WAI) and bowl life of breakfast cereals up to 20 min. High tannin bran addition increased the fiber contents and antioxidants level in cereals. Addition of high tannin bran decreased the expansion ratio and increased bulk density of cereals. These results were very encouraging. Therefore it was decided to use three types (white, black, high tannin) whole ground sorghum up to 85% without and with additional finely ground high tannin bran to develop breakfast cereals and snacks.

The main objectives of this study were

- Develop ready to eat gluten free breakfast cereals and snacks with acceptable sensory and nutritional characteristics by using whole ground sorghum with additional high tannin bran.
- Determine the effect of extrusion and different types of sorghum on total phenols, condensed tannins and antioxidant activity of extrudates.
- Determine the effect of sorghum extrudates on starch digestibility.

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2. LITERATURE REVIEW

2.1 SORGHUM

Sorghum bicolor (L.) Moench is known under a variety of names: milo, sorgo, sudangrass or sorghum in North America, great millet, feterita and guinea corn in West Africa, Kafir corn in South Africa, Dura in Sudan, Mtama, shallu in Eastern Africa, Jowar, Jwaarie, Jola, jawa, cholam, durra or Jondhahlaa in India, Jowar in Pakistan and Kaoliang in China (FAO, 1995; Purseglove, 1972). It is the fifth most important cereal crop grown in the world. It is used for food, fodder, production of alcoholic beverages and biofuels. Sweet sorghum syrup is called as molasses in parts of the U.S., although it is not true molasses. Most varieties of sorghum are drought and heat resistant and are especially important in arid regions. Sorghum has been a dietary staple for millennia in parts of India, Africa and China (O'Kennedy et al., 2006). Sorghum is an important component of pastures and fodder in many tropical regions.

The United States is the world's largest producer of sorghum followed by India and Nigeria (FAO, 2009). Sorghum and corn are comparable in costs of production and in nutrition, therefore the environment is the largest determining factor for choosing which to grow. Sorghum requires less water than corn, so is likely to be grown as a replacement to corn and produce better yields than corn in hotter and drier areas, such as the Southern US, Africa, Central America and South Asia (Sorghum, 2011).

The sorghum plant is composed of vegetative parts consisting of a fibrous root system, stem and leaves with leaf sheaths wrapping around the node and internode of the clum. The reproductive portion of sorghum is the panicle. The sorghum seed is a caryopsis or kernel composed of pericarp, endosperm and embryo (Figure 1). Each of these consists of complex sections and constituents. The sorghum kernel can be white, gray, red or brown in color, based on combination of pericarp color (which can be white or red only) and the presence and absence of testa (seed coat which is usually dark in color) (Obilana, 2004).

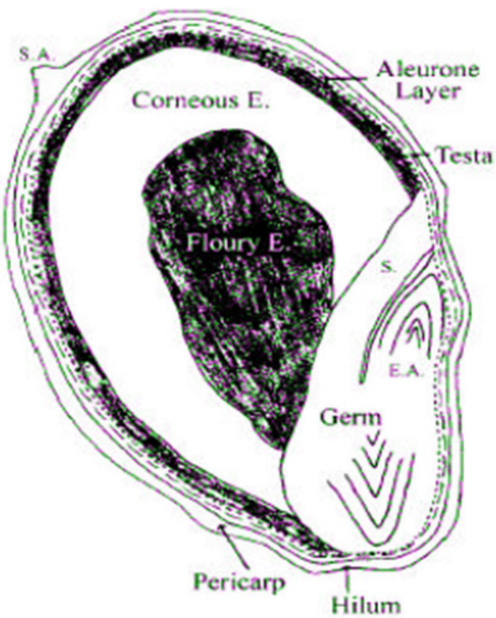


Figure 1: Sorghum caryopsis. Showing the pericarp endosperm (E) (aleurone layer, corneous, and floury), and germ [scutellum (S) and embryonic axis (EA)] (Source: Rooney and Miller, 1982)

Sorghum has several shades of red and brown color due to expressivity and intensifier gene action. The endosperm is white (yellow endosperm also exists) and can be corneous (hard and translucent) or floury (soft and opaque). The endosperm tissue of sorghum, like other cereals, is composed of the aleurone layer, peripheral, corneous and floury areas. The aleurone is the outer layer of the endosperm and consists of a single layer of rectangular cells with thick cell walls (Rooney & Sullins, 1973; Rooney & Miller, 1982).

There are three characteristic areas in the endosperm, peripheral, corneous (hard) and soft or floury. In the peripheral endosperm, there is a high protein content in the form of strong protein matrix and protein bodies, therefore it is extremely dense, hard, and resistant to water penetration, and digestion. Corneous (hard) endosperm has a continuous protein matrix that contains protein bodies dispersed throughout, with no air voids and with the starch physically surrounded by the protein. This factor also makes it translucent (Floyd, 1996). Floury (soft) endosperm in the center of the kernel has a discontinuous protein matrix and few protein bodies (Ring et al., 1988) and air cells that make it look opaque. The pericarp of sorghum grain originates from the ovary wall and is divided into many layers, the epicarp, mesocarp, and endocarp, with a pigmented seed coat lying underneath in some varieties. Unlike other cereals, the mesocarp of some sorghum varieties contains starch granules (Rooney & Waniska, 2001). The color of the sorghum pericarp is a combination of primarily anthocyanin and anthocyanidin pigments and other flavonoid compounds (Hahn et al., 1984). Some sorghum varieties have a pigmented seed coat (testa) that contains condensed tannins (Earp & Rooney, 1986; Earp et al., 2004)

The germ of sorghum kernel consists of two major parts: the embryonic axis and the scutellum. The embryonic axis contains the new plant and is divided into a radicle which develops into the primary roots and plumule which forms the leaves and stems. The scutellum is a single cotyledon and contains reserve nutrients which include moderate amounts of oil, protein, enzymes and minerals and serves as the bridge or connection between the endosperm and germ (Earp et al., 2004). Chemical composition of sorghum grain shows that it is a good source of protein, fiber and minerals (Table 1).

2.2 SORGHUM BRAN

Decortication is a term commonly used as synonymous with de-hulling and pearling the sorghum kernel. It involves the removal of pericarp and aleurone layers rich in fiber, ash, protein and fat, partial removal of the germ and ash (Rooney & Miller, 1982). This “bran” fraction is higher in dietary fiber and phenolic compounds (Rooney & Murty, 1982). Sorghum bran contains high levels of phenols, policosanols and sterols. The phenolic compounds serve as defenses for the sorghum against pests and diseases (Awika & Rooney, 2004).

Policosanol is the generic term for a natural extract of plant waxes. These are a mixture of high molecular weight aliphatic alcohols (Gouni-Berthold & Berthold, 2002). They are used as nutritional supplements to decrease LDL cholesterol and increase HDL cholesterol to help prevent atherosclerosis (Varady et al., 2003).

Table 1: Composition (%) of sorghum whole grain (dry wt. basis)

Component	Value	Range
Protein (N X 6.25)	11.6	8.1-16.8
Albumins	5.7	1.6-9.2
Globulins	7.1	1.9-10.3
Prolamins	52.7	39.3-72.9
Glutelins	34.4	23.5-45
Lipids	3.4	1.4-6.4
Ash	2.2	1.2-7.1
Nitrogen-free extract	79.5	65.3-81.0
Fiber		
Crude	2.7	0.4-7.3
Dietary, insoluble	7.2	6.5-7.9
Dietary, soluble	1.1	1.0-1.2
Acid detergent	3.3	2.9-3.6
Sugars	2.1	1.3-2.6

Source: Rooney & Waniska (2001)

In sorghum, wax comprises about 0.2% of the grain, generally higher than in other cereals and the policosanols represent 19–46% of it (Avato et al., 1990). Approximately 38–92 mg of policosanols are in 100g of sorghum grain (Awika & Rooney, 2004). Policosanols have cholesterol-lowering potency comparable to expensive and potentially harmful drugs (McCarty, 2002). Castano et al. (2002) reported that 10 mg/day of policosanol was more effective than 20 mg/day of lovastatin in reducing LDL cholesterol and raising HDL cholesterol levels. Various phytochemicals are available in sorghum depending on the variety of sorghum while phenolic acids and flavonoids are the most abundant. These phenolic acids and flavonoids are considered major antioxidants.

Most of the fiber is present in the bran (pericarp and cell walls). Sorghum contains 6.5-7.9% insoluble fiber and 1.1-1.2% soluble fiber mainly beta-glucans and pentosans. Fibers in cell walls of the aleurone and endosperm are associated with ferulic and caffeic acids. Fiber in the pericarp provides structural and protective functions, therefore fiber content of sorghum products depends on the extant of pericarp removal during milling (decortication). Insoluble dietary fiber increases during food processing due to increased levels of bound protein, mainly kafirins and enzyme-resistant starch. In tannin sorghum, cooking also forms polyphenol-protein complexes, which increases bulking ability (Waniska et al., 2004). Sorghum is a very good source of vitamins and minerals which are present in the aleurone layer and the germ. Sorghum aleurone layer is not a major source of endosperm-degrading enzymes (Waniska et al., 2004).

2.3 SORGHUM PROTEINS AND LIPIDS

Sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of poor people living in Africa, Asia, and the semi-arid tropics (Klopfenstein & Hoseney, 1995; Duodu et al., 2003). It contains approximately 10% protein (Subramanian & Jambunathan, 1982; FAO, 1995). There are four types of proteins in cereal grains but their ratios vary according to each cereal. These proteins are albumins, globulins, glutelins, and prolamins (Table 2). The prolamins, called kafirins, account for roughly 70-90% of the total sorghum grain protein (Hamaker et al., 1995).

Digestibility of sorghum protein decreases upon cooking, apparently through protein crosslinking (Duodu et al., 2003; Waniska et al., 2004). Protein contents and composition in sorghum vary due to agronomic conditions and genotype. Kafirins and glutelins comprise the major protein fractions in sorghum. These fractions are primarily located within the protein bodies and protein matrix of the endosperm, respectively. The alcohol soluble fraction comprises 50% of the protein. These proteins are hydrophobic, rich in glutamine, leucine, alanine and proline, contain little lysine and are primarily located within protein bodies. Kafirins contain cross-linked proteins that slow digestibility of the proteins. Sorghum identified with easier to digest proteins have protein bodies with a modified structure and contain less kafirins and less cross-linked protein after cooking. Glutelins are high molecular weight proteins, mainly located in the protein matrix. The lysine rich protein fractions, albumin and globulin predominate in the germ of sorghum. Lysine and threonine are the first and second most limiting amino acids in sorghum proteins. Sorghum lysine meets ~40% of the recommended level for infants (Waniska et al., 2004).

The germ of sorghum contains 80% of the ~3.5% lipid in the caryopsis. The fatty acid composition consists mainly of linoleic (49%), oleic (31%) and palmitic (14.3%) acids. Refined sorghum oil is very similar to maize oil in quality and fatty acid contents. The reduced oil contents of sorghum as compared to maize are an advantage for some applications in food products like extrusion of whole grain sorghum (Waniska et al., 2004). The proportionally large sorghum germ results in high oil content in the kernel, and this may lead to rancidity in the sorghum flour, therefore sometimes it is required to separate the germ from the kernel during milling (Taylor & Dewar, 2001).

Table 2: Compositional distribution of proteins in major cereal grains

Cereal grain	Albumin	Prolamins	Globulins	Glutelins
Barley	~12	25-52	8-12	52-55
Corn	4-8	47-55	3-4	38-45
Oats	10-20	12-14	12-55	23-54
Rice	5-11	2-7	~10	77-78
Rye	10-44	21-42	10-19	25-40
Sorghum	~4	~48	~9	~37
Wheat	9-15	33-45	6-7	40-46

Source: Pascoe & Fulcher (2007)

2.4 SORGHUM STARCH

The primary role of carbohydrates (sugars and starches) is to provide energy to cells in the body. They can be classified into two broad categories: available and unavailable. Available carbohydrates are those digested and absorbed by humans, and they include starch and soluble sugar. Unavailable carbohydrates, or dietary fibers, are not digested by the endogenous secretion of the human digestive tract (Southgate, 1995). Starch is the most abundant cereal polysaccharide, and it is a major food reserve that provides a bulk nutrient and energy source in the human diet. On a weight basis, 50 - 75% of the sorghum grain is starch (Rooney & Serna-Saldivar, 2003) located in the endosperm. Starch granules in sorghum range from 2-30µm in diameter. These are polygonal in corneous endosperm and are smaller, round in floury endosperm (Serna-Saldivar & Rooney, 1995). Sorghum starch is mainly composed of 70-80% amylopectin and 20-30% amylose. Waxy sorghum contains 100% amylopectin, it is very similar to the waxy starch of corn but the gelatinization temperature (71-80°C) is slightly higher than corn (Sweat et al., 1984). The major differences between corn and sorghum starches are the type and distribution of proteins surrounding the starch in the endosperm (Rooney & Waniska, 2001). Sorghum starch has sugars (Table 3) that are biologically equivalent to maize starch (Rooney & Riggs, 1971).

Sorghum starch from the hard endosperm is difficult to separate from protein matrix but wet milling of sorghum produces excellent prime starch (Waniska et al., 2004). Experience with livestock feeding (Riley, 1984) and brewing (Goode & Arendt, 2003) suggests that starch in whole sorghum grain may be slightly less digestible due to the hard peripheral endosperm layer limiting access to the interior (Rooney & Pflugfelder, 1986; Hamaker & Bugusu, 2003). Austin (2008) found that combined effects of tannins and anthocyanins in black with tannin sorghums significantly ($P<0.05$) decreased starch digestibility, estimated glycemic index, and increased resistant starch contents of whole sorghum grain and bran added to endosperm porridges even more than other specialty sorghum varieties.

Steam-flaking and reconstitution are effective in raising sorghum digestibility by breaking open the kernels and exposing the interior (Rooney & Pflugfelder, 1986). Palomino-Siller, (2006) indicated that condensed tannin sorghums may interfere with starch digestion after 60 min of incubation. Tannin sorghum porridges reached a steady state of starch digestion while white sorghum porridges had a higher percentage of starch digested. Toomey (1988) described that starches and sugars (Table 3) in sorghum are released more slowly than in other cereals ann it could be beneficial to diabetics.

Table 3: Sugar in mature sorghum kernels

Sugars	Range (%)
Soluble sugars	2.2-3.8
Free reducing sugars	0.9-2.5
Non reducing sugars	1.3-1.4
Glucose	0.6-1.8
Fructose	0.3-0.7

Source: Waniska et al., 2004

2.5 PHENOLIC COMPOUNDS AND STARCH DIGESTIBILITY

Polyphenols are a structural class of natural, synthetic and semisynthetic organic chemicals characterized by the presence of large multiples of phenol units. In cereals, phenolic compounds are found in both free and bound form. Free phenolic compounds located in the outer layer of the pericarp are proanthocyanidins or flavonoids, while the bound phenolic compounds present in the cell wall, are ester-linked to cell-wall polymers with ferulic acid and its dehydrodimer derivatives (Bonolia et al., 2004).

All sorghums contain phenolic compounds but some sorghum varieties have extremely high contents of phenolic compounds that aid in the natural defense of plants against pests and diseases. This makes sorghum unique because of the quality and quantity of phenolic compounds, including significantly high condensed tannins in some varieties (Butler, 1990). In sorghum these compounds are mainly located in the bran fraction. These grains have significant antioxidant properties (Awika & Rooney, 2004). Phenolic compounds identified in sorghum belong to three major groups: phenolic acids, polymeric flavonoids and simple flavonoids (Dykes & Rooney, 2006). Phenolic acids are derivatives of benzoic and cinnamic acids and are present in all cereals.

Flavonoids are compounds with a C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon link and these include flavanols, flavones, flavanones, flavonols and anthocyanins (Figures 2-6) (naturally occurring, water-soluble compounds), (Dykes & Rooney., 2007). The most abundant anthocyanins in sorghum grain are 3-deoxyanthocyanidins, e.g. apigeninidin 6 and luteolinidin (Dicko et al., 2006). Black sorghum has significantly ($P<0.05$) more anthocyanin pigments (4-9.8

mg/g) than other sorghums followed by red sorghum (3.3 mg/g), and brown sorghum (1.6-3.9 mg/g) on a dry weight basis (Awika & Rooney, 2004).

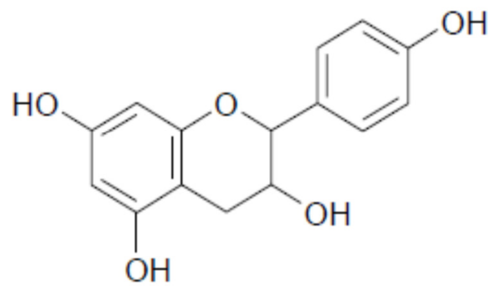


Figure 2: Chemical structure of flavanols

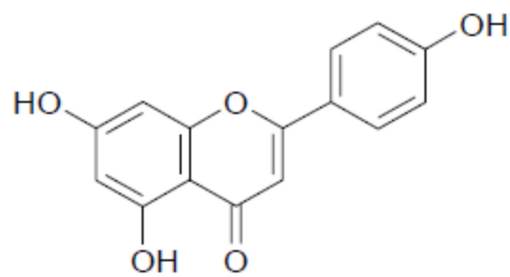


Figure 3: Chemical structure of flavones

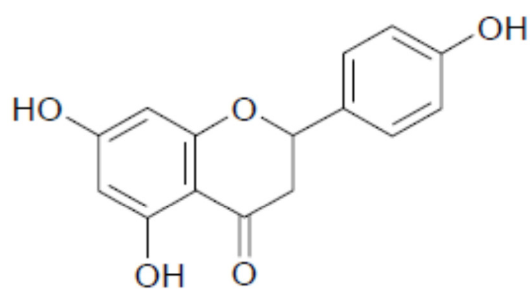


Figure 4: Chemical structure of flavanones

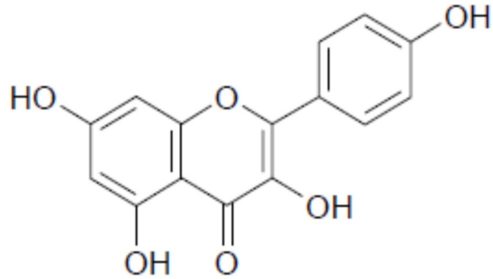


Figure 5: Chemical structure of flavonols

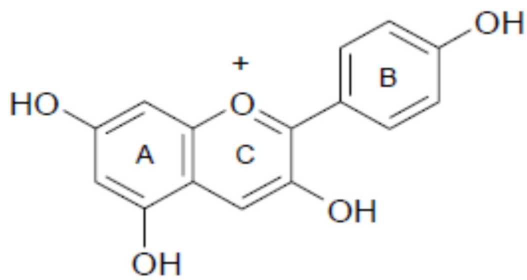


Figure 6: Chemical structure of anthocyanidins

In vivo studies showed that anthocyanins may help prevent obesity (Prior et al., 2007), diabetes (Jankowski et al., 2000), heart disease (Tsuda et al., 2003), inflammation (Lietti et al., 1976) and certain cancers (Karaivanova et al., 1990). Jankowski et al. (2000) reported that grape anthocyanins prevented the generation of free radicals, decreased lipid peroxidation, reduced pancreatic swelling, and decreased blood sugar concentrations in urine and blood serum. Purple corn anthocyanins inhibited the typical symptoms of hyperinsulinemia, and hyperleptinemia, which generally occur with high-fat diets (Tsuda, 2003).

Phenolic compounds may decrease the risk of diseases by lowering the amount of free radicals (Dykes et al., 2005). High tannin brans have high oxygen radical absorbance capacity values (Awika et al., 2003b). Free radicals are responsible for aging, tissue damage and possible cancer, arthritis and inflammatory bowel disease. Phenolic compounds present in sorghum complex with proteins (Haslam, 1996; Riedl & Hagerman, 2001) and carbohydrates (Asquith et al., 1987; Naczk et al., 2006) generating insoluble compounds. Sorghum protein digestibility decreases upon cooking, apparently through the formation of crosslinks (Duodu et al., 2003). Austin (2008) mentioned that black sorghum bran prevented anthocyanins from reducing estimated glycemic index of porridges when they were added to hard and soft endosperm flours. Whole grain sorghum porridges had significantly ($P<0.05$) lower starch digestibility and EGI values than whole white corn porridges.

2.6 CONDENSED TANNINS

Only sorghum cultivars with a pigmented testa produce condensed tannins or proanthocyanidins (Waniska, 2000) also called procyanidins. These are mainly polymerized products of flavan-3-ols and/or flavan 3 (Figure 7), 4-diol subunits that are deposited in the pigmented testa layer of sorghum kernels (Dykes & Rooney, 2007). The pericarp color of the sorghum kernel is controlled by the R and Y genes, which interact epistatically to produce red, yellow, and white pericarp colors (Rooney, 2000). A pericarp is white when the Y locus is homozygous recessive (rryy or R_yy); it is yellow in the presence of recessive alleles at the R locus and at least one dominant allele at the Y locus (rrY_).

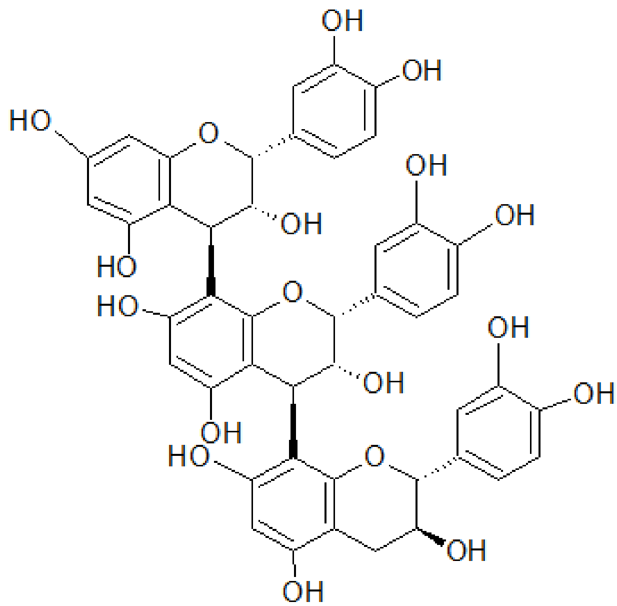


Figure 7: Chemical structure of polyflavan-3-ol

When both R and Y loci have a dominant allele (R_Y_), the pericarp is red. In tannin sorghum, the pigmented testa is controlled by the complementary B1 and B2 genes (Ring et al., 1988; Dykes et al., 2005). The spreader gene (S) controls the presence of pigments and possibly tannins in the epicarp. When S is dominant, more tannins are in the pericarp and testa layers (Dykes et al., 2005). Tannins present in different fruits and cereals are strong antioxidants and protect plants against insects and diseases (Awika, 2003). Although tannins are considered anti-nutritional compounds, tannin sorghums have been consumed and preferred for centuries as breads, porridges, and alcoholic beverages in Africa and Asia. Awika & Rooney (2004) stated that tannin sorghums should be considered a source of natural antioxidants, dietary fiber, and color compounds (Table 4). Tannins bind proteins, carbohydrates and minerals, which decrease digestibility of nutrients and reduce the feed efficiency of ruminants and monogastrics (Waniska & Rooney, 2000; Dykes & Rooney, 2006). These sorghum compounds may have anti-carcinogenic, cardiovascular, gastro-protective, anti-ulcerogenic, cholesterol- lowering properties and they also promote urinary tract health (Dykes & Rooney, 2007).

Expanded-snacks, made with tannin sorghum, retained 89% of their original whole-grain antioxidant potential (Awika, 2003). Palomino-Siller (2006) reported that the addition of 12% tannin bran to a bread formulation significantly ($P<0.05$) decreased starch digestibility and EGI values. In a similar study Austin (2008) found hi-tannin and black sorghum with tannins were most effective for lowering starch digestibility, estimated glycemic index and increasing resistant starch contents of porridges.

Table 4: Tannin contents of sorghum grain and other cereals

Commodity	mg/g (dry wt)
Tannin sorghum	10-68
Tannin-free sorghum	0-0.5
Finger millet	3-13
Buck wheat groats	0-1.7

Source: Awika & Rooney (2004)

2.7 GLYCEMIC INDEX (GI)

GI is a ranking of carbohydrates based on their immediate effect on blood glucose (blood sugar) levels. It is measured by comparing the increase in blood sugar after eating 50 grams of available carbohydrate from a single food with the increase in blood sugar after eating the same quantity of available carbohydrate in either glucose or white bread (Jenkins et al., 1981). The GI can be a useful indicator of starch digestion of foods. A food that is easily broken down during digestion and quickly absorbed gives high blood glucose response. Foods are classified as low (<55), intermediate (55-70) and high GI (>70) with glucose as the reference standard. Higher values are found in more highly processed foods, while whole grain foods have lower values (Wolever and Bolognesi, 1996). Consuming low GI foods helps to prevent extreme blood glucose changes and to slow absorption of carbohydrates (Price & Butler, 1977). Lowering postprandial blood glucose (by consuming low GI foods) has positive health outcomes for both healthy subjects and patients with insulin resistance (Lang & Vitapole, 2004).

2.8 EFFECTS OF EXTRUSION ON PHYTOCHEMICALS

Awika (2003) found that extruded tannin and black sorghum had high retention of antioxidant activity. Black sorghum extrudates showed retention levels for anthocyanins up to 54%, phenols up to 72% and antioxidant activity levels up to 81% (Awika, 2003). Blueberry and grape anthocyanins were significantly reduced by extrusion and by ascorbic acid in sweetened corn breakfast cereals (Chaovanalikit, 1999). According to Camire (2001), total free phenolics, were lower after extrusion.

Higher barrel temperature and feed moisture protected free phenolics. While polymerization and browning contributed to anthocyanin losses. Awika (2003) mentioned that extrusion cooking significantly reduced ABTS antioxidant activity by 83 to 89%, for sorghum products compared to the raw grains. This was expected since measurable total phenols and tannins were also significantly reduced. Thus, only 13 to 23% of antioxidant activity of the original raw or unprocessed grain was extracted from the product. Ngwenya (2007) reported that the total phenols were significantly affected by sorghum type, decortication and processing (Table 5). The sorghum types with pigmented testa layers (condensed tannins) had higher levels of total phenols [19.7 to 24.5 mg catechin equivalents per g (mg CE/g)], than the types without a pigmented testa (2.7-5.3 mg CE/g). The total phenol levels differed significantly ($P<0.05$) between the different sorghum types. The tannin sorghums had the highest, followed by NK 283 and lastly Macia.

Table 5: Effect of extrusion cooking on total phenols of sorghum

Sample	Raw grain whole	Raw grain decorticated	Extruded sorghum whole	Extruded sorghum decorticated
Macia	2.7k	2.2l	1.8l	2.31l
NK 283	5.3j	2.0l	2.3k	3.4j
Red Swazi	19.7c	6.6h	6.0h	2.5k
NS 5511	22.4b	4.7j	6.7h	4.1j
Framida	24.5a	8.5g	5.3i	3.7j

Total phenols expressed as mg Catechin equivalents per g sample, dry basis (Folin Ciocalteu method).
Values with different letters are significantly different at P<0.05
(Source: Ngwenya, 2007)

2.9 SORGHUM AS GLUTEN FREE CEREAL

Celice disease is a syndrome characterized by damage to the mucosa of the small intestine caused by ingestion of certain wheat proteins and related proteins in rye and barley (Fasano & Catassi, 2001). This disease is triggered by ingesting gluten proteins which are naturally present in some cereal grains. These proteins are gliadins (Kagnoff et al., 1982) and glutenins (Van de Wal et al., 1999) of wheat and have been shown to contain protein sequences that are not tolerated by celiacs. While CD can’t be cured, its symptoms can be managed through diet only. This means that wheat, rye, and barley have to be avoided, including durum wheat, spelt wheat, kamut (khorasan wheat), einkorn, and triticale (Kasarda, 2001; Kasarda & D’Ovidio, 1999).

Sorghum is recommended as a safe food for celiac patients, because it is only distantly related to the triticeae tribe cereals (Kasarda, 2001), being a member of the panicoideae sub-family which also includes corn and most millets (Shewry, 2002). Sorghum therefore, provides a good basis for gluten-free breads and other baked products like cakes and cookies, crackers, breakfast cereals and snacks. The development of white food-grade, sorghum lines has enabled white, bland-tasting flour production from sorghum grain. This flour is useful in food products because it does not impart unusual colors or strong flavors. It may be desired over maize flour for these reasons (Waniska & Rooney, 2002). Whole grain sorghum flour is a wholesome, hearty grain that provides fiber with a mild flavor that does not compete with the delicate flavors of other food ingredients (Rees & Henneman, 2009)

2.10 SORGHUM BASED GLUTEN FREE RTE CEREALS AND SNACKS

Ready-to-eat (RTE) breakfast cereals are processed grain formulations suitable for consumption without any further cooking at home. These are relatively shelf stable, light weight and favored by consumers of all ages because of their convenience, variety, and high nutritional value (Hegenbart, 1995). There are three types of breakfast cereals; ready to eat cereals, hot cereals and cereal bars available in the market. Ready to eat breakfast cereals include cold cereals, mostly corn, wheat, rice, and oat based and are combined with milk for consumption. Hot cereals, such as oatmeal porridges are traditionally consumed in American households at breakfast (IBIS World Industry report, 2007).

A large number of gluten-free products have been made with combinations of sorghum and other acceptable gluten-free cereals. These products usually use tapioca and/or potato starches, while some baked products are made by using decorticated sorghum flour. Sorghum does not adversely affect taste and provides acceptable starch that interacts with the tuber starches and hydrocolloids to produce relatively good breads (Asif et al., 2010). The white food sorghums are processed into flour and other products, including expanded snacks, cookies, bread and ethnic foods, and are gaining popularity in areas like Japan (Awika & Rooney, 2004).

Sorghum based, gluten free breakfast cereals, comparable to commercially available corn-based breakfast cereals in physical and functional properties were developed with twin screw extruder. Whole ground white sorghum with the ratio of 50 and 60% with 5 and 10% high tannin sorghum bran was used while other ingredients were corn flour, rice flour and corn starch. Bulk density of these cereals was 0.13 kg/L and 0.17 kg/L respectively. The bowl life was 12 and 18 min respectively. Extrusion of the whole ground high tannin and black sorghum produced strong dark brown extrudates which may reduce levels of cocoa required in finished cocoa flavored products like cocoa puffs (Asif et al., 2009).

Different types of extruders can be used for the development of gluten-free breakfast cereals and snacks. The type of extruder depends upon the desired final product and initial investment. Dry extruders require relatively low capital investment to produce direct expanded sorghum snacks with different levels of decortication. Decorticated white sorghum can be used to produce snacks comparable to corn meal puffed snacks. A short-barrel, friction-type extruder is a good choice where steam is not available. Dry extruders are able to grind whole sorghum during extrusion. The size of the product may be limited. The high pressure involved with this type of extruder makes it extremely difficult to shape a product that is less than 2 mm (Asif et al., 2010).

Cereals and snacks can be prepared using sorghum with other cereals and ingredients by using a single-screw wet extruder. These extruders are easy to operate and cost more than the dry extruder but are about half the price of twin-screw extruders. Single-screw wet extruders yield superior shaped products compared with dry extruders because of better process control. Twin-screw extruders can be used to develop versatile breakfast cereals and snacks by using sorghum with high levels of bran and additional ingredients. These handle a wide range of particle sizes in ingredients. Finely ground sorghum and bran can be fed directly into the twin screw extruder, as well as very coarse ground sorghum with other ingredients (Waniska et al., 2004).

3. MATERIALS AND METHODS

3.1 SORGHUM AND OTHER INGREDIENTS

White food-type sorghum (Atx635*RTx436, College Station 01), High-tannin sorghum (CSC3*R28, College Station) and Black sorghum (A0530/BlackTX430, College Station), were used in this research. All sorghums were (Figure 8) obtained from the Texas Sorghum Breeding and Genetics program. Sorghum grains were cleaned, de-glumed and stored at below 4°C until used.

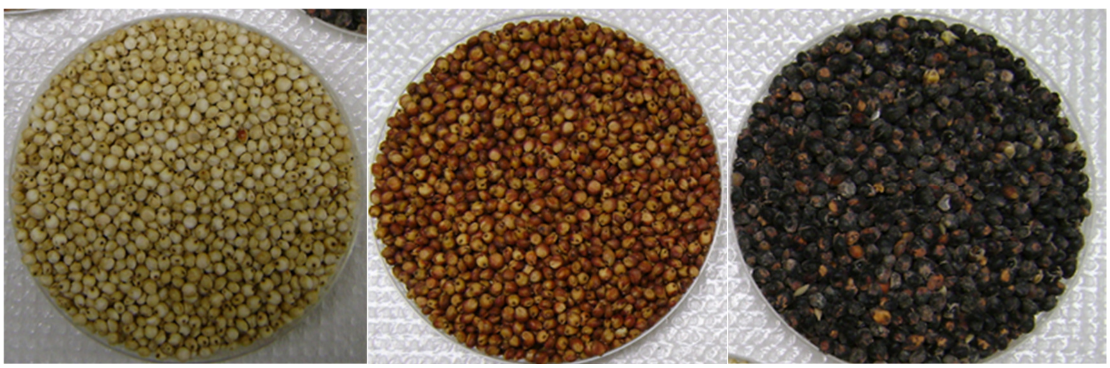


Figure 8: White, high tannin and black sorghum whole grains

Yellow corn flour and soy isolates were purchased from Archer Daniels Midland (ADM) milling. Hard white whole wheat flour was obtained from ConAgra mills. Oat flour was purchased from Can-Oat milling (Canada). Whole rice and corn starch, cane sugar and rock salt were purchased from local markets (HEB, San Antonio, TX). High tannin bran (30% decortication) was obtained from ADM. All the ingredients were stored at below 4°C until used.

3.2 PHYSICAL CHARACTERISTICS OF SORGHUM

3.2.1 Single Kernel Hardness Test

White, high tannin and black sorghum kernels were characterized for hardness and diameter by using a single kernel hardness tester (SKHT, model SKCS 4100, Perten Instruments, Reno, NY).

3.2.2 True Density

Density of all three types of sorghum was measured using a gas-comparison pycnometer (Multipycnometer, Quantachrome, Syosset, NY).

3.2.3 Thousand-Kernel Weight (TKW)

TKW was performed by weighing 100 kernels of each type of sorghum and multiplying by 10.

3.2.4 Color

Color of sorghum grains and blended flours were measured with a colorimeter (model CR-310, Minolta, Osaka, Japan) using CIE L* a* b* color values.

3.3 MILLING OF SORGHUM AND HIGH TANNIN BRAN

3.3.1 Milling

Whole sorghum from all varieties, bran whole rice kernel was milled by using a hammer mill (Fitzmill comminutor, Fitzpatric, Elmhurst, IL) fitted with 0.0027 inch (69 micron) screen. Milled sorghum was stored in labeled buckets with a tightly fitted lid at 4°C until used.

3.3.2 Particle Size Distribution of Whole Ground Sorghum

Particle size distribution of whole ground sorghum flour was determined using U.S. standard testing sieves (Seedburo Equipment company, Chicago, IL) numbers 20, 40, 60, 80, and 100 for percentage retained above each sieve were recorded. Analyses were performed in triplicate.

3.4 MIXING OF INGREDIENTS AND EXTRUSION

3.4.1 Ingredient Blending

A total of four experiments were conducted to determine the use of sorghum in breakfast cereals and snacks in different combinations. The first three experiments were preliminary and the fourth was the final experiment. In the preliminary experiments the objective was to optimize the sorghum and high tannin bran usage. In the first experiment, whole ground high tannin sorghum and high tannin sorghum bran was mixed with gluten containing ingredients to develop breakfast cereals rich in dietary fiber with enhanced antioxidant activity.

In the other two preliminary experiments, gluten free breakfast cereals and snacks were developed by using different levels of whole ground sorghum grains. High-tannin whole ground sorghum with high-tannin sorghum bran was used in the first experiment, other ingredients were corn flour, hard wheat flour, and oat flour (Table 6).

A total of 13 treatments were designed using the ingredients mentioned. The objective was to optimize the use of whole ground high tannin sorghum and high tannin bran in extrudates.

In the second experiment, white whole ground sorghum was used and for this, two treatments were designed. In the first treatment, 50% sorghum and 5% high tannin sorghum bran and second treatment 60% sorghum and 10% high tannin sorghum bran was used. Other ingredients used in these treatments are given in Table 7.

In the third experiment, 80% of whole ground sorghum (white, black, and high tannin) and other ingredients were mixed according to ratios given in Table 8. In the fourth experiment 85% of whole ground sorghum (white, high tannin, black) with high tannin sorghum bran (0-6%) were blended using 12 treatments with two replications (Table 9). The formulation in experiment 4 contained rice flour, corn starch, sugar and salt, all were mixed and each treatment weighed 6.8 kg (15 Ib).

Startup material (control) in first and second experiments was 100% yellow corn flour. In third experiment 80% yellow corn flour and 20% other ingredients (Table 8) while in the fourth experiment, 85% yellow corn flour and 15% other ingredients (Table 9) were used as control. All ingredients were mixed in a ribbon blender for ten min and stored at room temperature for 12 hrs.

3.4.2 Extrusion

A twin screw extruder (Wenger TX52, Wenger Manufacturing Inc. Sabetha, KS) was used to extrude the sorghum containing formulations. Processing conditions are given in Table 10. The configuration of the twin screw extruder is given in Figure 9.

3.4.3 Drying

After extrusion, extrudates were dried in hot air dryer (Wenger Manufacturing Inc. Sabetha, KS) at 105°C for 6 min, cooled for 6 min and packed in labeled air tight containers for further analysis.

3.4.4 Sugar/Cheese Seasoning Coating

Extrudates from selected treatments were coated with sugar solution and cheese slurry. For the sugar coating, 100g of sugar solution (65% sugar) was applied to 250g of extrudates in a coating pan. For cheese coating, the slurry was prepared by mixing 45% of cheese seasonings (Spray Dynamics, St. Clair, MO) and 55% of vegetable oil (Soybean oil). The 40g of cheese slurry was sprayed on 60g of dry extrudates.

Table 6: Ready to eat breakfast cereal formulation (%) with sorghum, bran and other ingredients (Experiment 1)

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
Corn flour	42	32	30	28	22	20	18	12	10	8	2	0	0
Sorghum*	0	10	10	10	20	20	20	30	30	30	40	40	40
Hard wheat flour	30	30	28	28	30	28	28	30	28	28	30	28	26
Oat flour	20	20	20	18	20	20	18	20	20	18	20	20	18
Sugar	7	7	6	5	7	6	5	7	6	5	7	5	5
Salt	1	1	1	1	1	1	1	1	1	1	1	1	1
Bran**	0	0	5	10	0	5	10	0	5	10	0	5	10
Total	100	100	100	100	100	100	100	100	100	100	100	100	100

*High-tannin whole ground sorghum
**High-tannin sorghum bran

Table 7: Gluten free, ready to eat breakfast cereals and snacks formulation (%) with whole ground white sorghum and high tannin sorghum bran (Experiment 2)

Ingredients	Treatment 1 (%)	Treatment 2 (%)
Whole ground white sorghum	50	60
High-tannin sorghum bran	5	10
Yellow corn flour	24	9
Sugar	7.5	7.5
Rice flour	6	6
Corn starch	4	4
Soy isolates	3	3
Salt	0.5	0.5
Total	100	100

Table 8: Gluten free, ready to breakfast cereals and snacks formulation (%) with whole ground white, high tannin and black sorghum (Experiment 3)

Ingredients	percentages
Whole ground sorghum (white, black, high tannin)	80
Rice flour	6
Corn flour	5
Soy isolates	5
Sugar	3
Salt	1

Table 9: Gluten free, ready to eat breakfast cereals and snacks formulations (%) with whole ground white, high tannin, black sorghum and high tannin sorghum bran (Experiment 4)

Treatments	Sorghum*(%)	Bran**(%)	Corn flour (%)	Rice flour (%)	Corn starch (%)	Sugar (%)	Salt (%)
Control	0	0	85	8	5	1.5	0.5
1	85	0	0	8	5	1.5	0.5
2	85	2	0	7	4	1.5	0.5
3	85	4	0	6	3	1.5	0.5
4	85	6	0	5	2	1.5.	0.5

*Whole ground white, high tannin and black sorghum
**High tannin sorghum bran

Table 10: Processing conditions of twin screw extruder (Wenger TX52)

Process	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Feed screw speed (rpm, kg/hr)	10-11 rpm, 40kg/hr	11 rpm, 42 kg/hr	11 rpm, 42 kg/hr	11 rpm, 42 kg/hr
Pre-conditioner speed (rpm)	205-222	306	305	306
Extruder screw speed (rpm)	308-406	309	411	310
Extruder motor load (%)	31-37%	25-35%	42%	45%
Extruder water flow (kg/hr)	0.7-4.3	4.1	1.1	4.1
Die diameter (mm)	2 mm (2 openings)	3 mm (2 openings)	3 mm (2 openings)	3 mm (2 openings)
Knife drive speed (%)	70-90%	Max	Max	Max
Barrel temperature zone 1 (set/actual) °C	70/32	50/39	50/42	50/40
Barrel temperature zone 2 (set/actual) °C	70/41	75/47	75/55	75/50
Barrel temperature zone 3 (set/actual) °C	70/70	75/75	75/74	75/75
Barrel temperature zone 4 (set/actual) °C	90/90	75/75	75/75	75/76
Barrel temperature zone 5 (set/actual) °C	90/110	95/120	90/112	95/118
Barrel temperature zone 6 (set/actual) °C	110/121	110/111	110/110	110/115

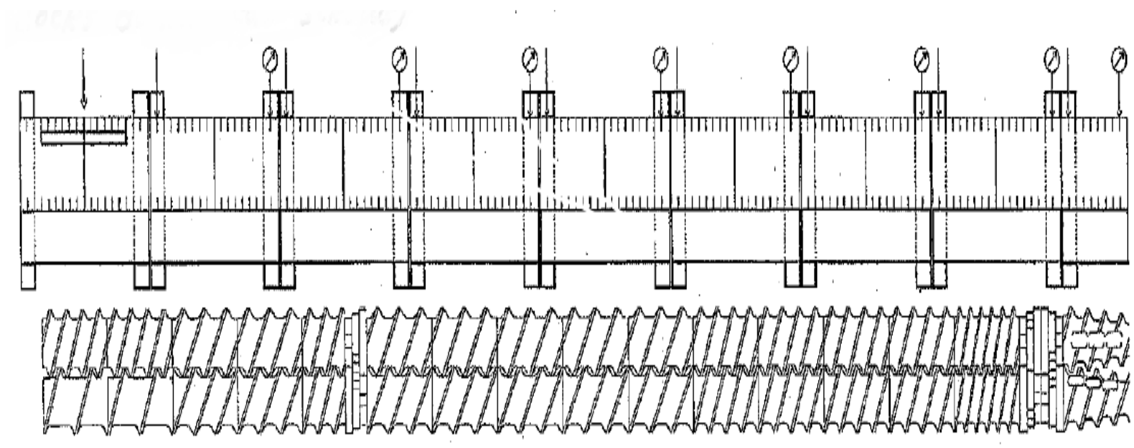


Figure 9: Screw configuration for twin screw extruder

3.5 PHYSICAL ATTRIBUTES OF EXTRUDATES

3.5.1 Color

Color of extrudates was measured with a colorimeter (model CR-310, Minolta, Osaka, Japan) using CIE L* a* b* color values.

3.5.2 Bulk Density (BD)

BD of extrudates was determined by taking a ratio of weight and volume. Volume was measured in 15L container and following formula was used:

$$\frac{\text{Weight of extrudates (g)}}{\text{Volume of Extrudates (L)}}$$

3.5.3 Expansion Ratio (ER)

Extrudates radial expansion was measured in mm with the use of a digital vernier caliper divided by the die diameter.

3.5.4 Extrudate Hardness

Maximum compression force (N) required to compress extrudates of sorghum up to 7 mm with a probe speed of 0.5 mm/sec., was measured with a texture analyzer model TA. XT2i (Texture Technologies Corp., Scarsdale, NY) fitted with a 25 Kg load cell.

3.5.5 Bowl Life

Bowl life of breakfast cereals in milk was measured by using the method described by Gregson & Lee (2003) with modifications. Four panelists were selected from graduate and undergraduate students attending Texas A&M University and were trained for texture profile for crispness by using the standard crispness scale mentioned by Meilgaard et al. (2007). Panelists were trained only for crisp and mushy texture of breakfast cereals keeping in view the definition of bowl life which is ‘the length of time that a breakfast cereal retains its crispness while immersed in milk’. Standards used for training of crisp texture were “Cheerios oat cereals” however for mushy texture “Quaker traditional oat” cooked and then cooled at room temperature were used. After 4 sessions of training, each member of the panel was presented with a stop-watch, 50g of cereals in a bowl and 200 mL of milk (1% fat). The panelists were instructed to simultaneously start the stop watch, add the milk, and then proceed to assess the texture of the cereal after every one minute by eating a few extrudates at a time. Each panelist noted the time at which the extrudates lost their crispy texture and repeated the assessment until satisfied that they had a bowl life. Data obtained was statistically analyzed to find the difference between bowl life of cereals.

3.6 SENSORY EVALUATION OF EXTRUDATES

Selected treatments with sugar coated cereals were used for consumer sensory evaluation. Consumers (n=55) were recruited from Texas A&M University staff and students by advertising at different departmental notice boards. Age, sex and ethnicity of consumers were required for demographic purposes. Some consideration was given to those who consume breakfast cereals at least twice a week. Panelists were told not to smoke on the day of evaluation and not to drink coffee/tea or eat/drink anything except water within 1 hour of evaluation.

One testing location was selected (Food Protein R&D Center's conference room) because it was a central location, easy to reach and had a parking facility. The testing area was completely odor free and separate from other food preparation areas. Sample preparation was done in a kitchen, away from testing areas. The testing location was away from offices, close to the entrance with sufficient shade free white light. The room was centrally air conditioned at a temperature of 22-24°C. A test plan was designed (Appendix A) covering sample handling, storing and presentation including three digit coding. Cereals were kept at room temperature while milk was refrigerated. Samples (about 40g each) were presented in styroform bowls with spoons while milk was presented in a styrofoam cup (250 mL). Double distilled de-ionized water, ricotta cheese and saltine crackers were provided to each panelist to rinse their palates between sample evaluations.

Presentation of the samples was randomized and coded with a three digit number to prevent bias related to "order of presentation". Seating in the room was arranged so they were unable to discuss or see the impression of other panelists. Panelists were provided a detailed orientation regarding number of samples to evaluate, time consumed, how to taste cereals and to complete the ballot.

The objective was to identify the degree of liking of products, therefore acceptance questions were included in this evaluation. The ballot (Appendix B) contained questions about appearance, color, taste/after taste, texture and overall acceptability. They were evaluated using a hedonic scale from 1 to 9. Scale 1 was for dislike extremely and 9 for like extremely. Null hypothesis ($H_\circ$) for consumer sensory evaluation was “all six types of ready to eat breakfast cereals were similar in appearance, color, taste/after taste, texture and overall acceptability” i.e. ($H_\circ$: $\mu_1 = \mu_2 = \ldots\ldots\ldots\mu_6$). While the alternative hypothesis (Ha) was that “at least two types of breakfast cereals were different in appearance, color, taste/after taste, texture and overall acceptability” i.e. (Ha: $\mu_1 \neq \mu_2 \neq \ldots\ldots\ldots\mu_6$).

3.7 CHEMICAL ANALYSIS OF EXTRUDATES

3.7.1 Moisture

Moisture contents of extrudates were determined by the moisture air oven method (AACC Int., Method 44-19.01) in triplicate.

3.7.2 Starch

Total starch was determined using a starch assay kit (Megazyme Int., Ireland, AACC Int. Method 76-13.01).

3.7.3 Water Absorption (WAI) and Water Soluble Index (WSI)

WAI is the weight of gel obtained per gram of dry sample. It was measured by method described by Anderson (1982) for measuring the swelling power of sorghum starch and bran containing extrudates. A one gram of ground extrudates (<40 mesh) was suspended in 10mL of distilled water in a 15 mL tared centrifuge tube and stirred for 30 min at 30°C. Subsequently, the dispersion was centrifuged at 3000 rpm for 20 min. The supernatant liquid was poured carefully into a tared evaporating dish, weighed and placed in an oven at 110°C overnight to completely evaporate the water. The remaining gel was weighed and WAI was calculated.

As an index of water solubility, the amount of dried solids recovered by evaporating the supernatant from the water absorption test was expressed as percentage of dry solids.

The following formulas were used to calculate WAI and WSI:

$$\text{WAI} = \frac{weight\ of\ sediment\ (gel)}{weight\ of\ dry\ solids}$$

$$\text{WSI} = \frac{weight\ of\ dissolved\ solids\ in\ supernatant}{weight\ of\ dry\ solids} \text{ X } 100$$

3.8 TOTAL PHENOLS, ANTIOXIDANT ACTIVITY AND TANNIN CONTENTS OF SORGHUM AND EXTRUDATES

3.8.1 Extraction for Total Phenols and Antioxidant Activity

All samples of sorghum grain, composite flour and extrudates were ground through a cyclotec mill (UDY Corp., Fort Collins, CO) (0.5 mm mesh) prior to extraction. For all assays three replicates (0.1-0.5g) were extracted in 25 mL of 1% HCl/methanol (v/v) for two hours while shaking at low speed in an Eberbach shaker (Eberbach Corp., MI). All extracts were centrifuged at 2790*g* for 10 min in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE) and decanted. To avoid oxidation, extracts were stored in the dark at -20°C and analyses were performed within 24 hours.

3.8.2 Extraction for HPLC Analysis

The extraction of flavonoids was performed as described by Dykes (2008). Three replicates of ground samples (1g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours while shaking at low speed using an Eberbach shaker. The extracts were centrifuged at 2790*g* for 10 minutes and then decanted. A second set of extracts were prepared for flavanone analysis. Samples (1g) were extracted in 10 mL of 1% HCl/methanol (v/v) for two hours in an Eberbach shaker. Each supernatant was transferred to glass tubes, sealed, and placed in a water bath for 90 min at 80ºC; after equilibration at room temperature all extracts were filtered using a 0.45 µm nylon membrane filter (Whatman Inc., Maidstone, UK) prior to HPLC analysis.

3.8.3 Total Phenols Analysis

Total phenols of the acidified methanol extracts were measured using the modified Folin-Ciocalteu method of Kaluza et al. (1980). One aliquot of the extract (0.1 mL) was dissolved in 1.1 mL of water and reacted with 0.4 mL of Folin-Ciocalteu reagent and 0.9 mL of 0.5*M* ethanolamine. The reaction was allowed to stand for 20 min at room temperature and the absorbance was read at 600 nm.

3.8.4 Antioxidant Activity Analysis

Antioxidant activity of sorghum extracts were measured *in vitro* by the ABTS assay. The $ABTS^{\cdot+}$ was obtained by reacting 3 mM of $K_2S_2O_8$ with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The $ABTS^{\cdot+}$ solution was then diluted with a pH 7.4 phosphate buffer (50:42.5:9.5; water:0.2 M Na_2HPO_4:0.2 M NaH_2PO_4) solution containing 150 mM NaCl (PBS) to obtain an initial absorbance of 1.5 at 734 nm. Fresh $ABTS^{\cdot+}$ solution was prepared each day of analysis. Dilutions of Trolox in methanol were used to prepare the standard curve. Samples and standards (100 μm) were reacted with the $ABTS^{\cdot+}$ solution (2900 μm) for 15 min.

3.8.5 HPLC Analysis

Extracts were analyzed on an Alliance 2695 system (Waters Corp., Milford, MA) with a Waters 996 photodiode array detector (PDA). Sorghum flavonoids were separated using a Luna C18 column (150 mm x 4.6 mm i.d., 5 μm) from Phenomenex (Torrance, CA). Column temperature was conditionated at 35 ºC. Injection volume was 20 μL. The mobile phase consisted of 4% formic acid in water (v/v) (Solvent A) and acetonitrile (Solvent B). The solvent flow rate was 1.0 mL/min. The 3-deoxyanthocyanins were separated using the following gradient: 0-20 min., 12-20% B; 20-40 min., 20-50% B; 40-50 min., 50% B. Flavones and flavanones were separated using the following gradient: 0-45 min., 15-41% B; 45-50 min., 41% B. The 3-deoxyanthocyanins, flavones, and flavanones were measured at 485 nm, 340 nm, and 280 nm respectively (Dykes, 2008). Identification of sorghum flavonoids was based on commercial standards' retention times, UV-Vis spectra, and LC-MS data. Quantification of each compound was done by comparing peak areas with that of a standard curve of each authentic standard. Molecular weight correction factors were used to quantify 5-methoxyluteolinidin and 7-methoxyapigeninidin (Chandra et al., 2001; Wu & Prior, 2005). Data was collected and processed using the Empower software version 1.0 (Waters Corp., Milford, MA).

3.9 *IN VITRO* STARCH DIGESTIBILITY OF EXTRUDATES

In vitro starch digestibility was determined by the Approved Method 32-40 (AACC International) modified by Chung et al. (2008). Ground extrudate samples (100 mg) were incubated with pancreatin (10 mg) and amyloglucosidase (12 U) in 4 mL of 0.1*M* sodium maleate buffer (pH 6.0) at 37°C with continuous shaking (200 strokes/min) for 0.5–16 hrs. After incubation, ethanol (95%) was added to inactivate the enzyme and the sample was centrifuged at 2,000 rpm for 10 min. Glucose content of the supernatant was measured by a glucose oxidase-peroxidase assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were those digested within 0.5 hr and at 0.5–16 hrs, respectively.

3.10 STATISTICAL ANALYSIS

One way ANOVA was used to determine a significant difference ($P<0.05$) between different types of sorghum extruded, antioxidant activity before and after extrusion, starch digestibility, and consumer acceptability of breakfast cereals and snacks. All values were expressed as means $\pm$ standard deviation for the three replicates. Pearson's correlation was used to determine relationship between sorghum types and starch digestibility and sorghum phenols, antioxidant activity and starch digestibility before and after extrusion. Sorghum types and overall liking of breakfast cereals and snacks were analyzed by using general linear modeling option. Statistical analysis was performed by using SPSS version 15 (SPSS Inc. Chicago).

4. RESULTS AND DISCUSSIONS

4.1 PHYSICAL PROPERTIES OF SORGHUM GRAINS

Thousand kernel weights of the sorghum varieties were significantly ($P<0.05$) different. Kernels of white (2.2 mm, 27 mg) and black (2.5 mm, 26 mg) sorghum varieties were significantly ($P<0.05$) bigger and heavier than those of high tannin (1.7 mm, 14.8 mg) sorghum (Table 11). High tannin kernels were lighter and smaller in diameter than that of white and black sorghum grains.

Kernel hardness values of sorghum varieties ranged from 58.3-85.3 measured with the SKHT (Table 11). The white sorghum variety had the hardest kernels with hardness index (HI) of 85.3±0.86, black sorghum was soft with an HI of 58.7±2.1. The HI of high tannin sorghum grain was 61±1. Black sorghum kernels contained a large amount of soft, floury endosperm while white sorghum kernels had a large portion of corneous endosperm. On the other hand high tannin sorghum contained an intermediate mixture between the two types. These factors were also reflected in the grains true densities. True density of white (1.35±0.03 g/cm^3) and black (1.35±0.03 g/cm^3) sorghum varieties were similar while high tannin sorghum had significantly ($P<0.05$) lower density (1.29±0.09 g/cm^3) than the other varieties (Table 11). White and black sorghum kernels were significantly larger and harder compared to high tannin sorghum kernels.

Table 11: Physical characteristics of white, high tannin and black sorghum grains

Sorghum Variety	Thousand Kernel Weight(g)	True Density (g/cm^3)	Kernel Diameter (mm)	Single Kernel Hardness Test (SKHT)
White (Atx635*RTx436)	27.8c	1.35b	2.2b	85.3b
High Tannin(CSC3*R28)	14.9a	1.29a	1.7a	61.0a
Black (A0530/BlackTX430)	26b	1.35b	2.5c	58.8a

SKHT = Hardness Index (-20 to 120) by Single Kernel Hardness Tester
Data values with different letters are significantly different at P<0.05

4.2 COLOR OF SORGHUM AND HIGH TANNIN SORGHUM BRAN

Color of all three whole ground sorghum and high tannin sorghum brans were significantly ($P<0.05$) different. The L* value of high tannin sorghum bran (60.3) and ground black sorghum (63.3) were lowest, it means that these were the darkest in color. Whole ground white sorghum had the highest L* value (88.2), indicating that it had the lightest color (Table 12). The high tannin sorghum bran was 30% decorticated fraction of high tannin sorghum contained some endosperm fractions. All the sorghum samples had significantly ($P<0.05$) different a* values and were more red than green. White sorghum had the lowest a* value (2.6) while high tannin sorghum bran had the highest (11.8). The b* value was positive for all samples which mean they were more yellow than blue. Whole ground white sorghum had the highest b* value which means it is more yellow. The L* values increased as a* value increased in all samples except high tannin bran (Figure 10).

Table 12: Color (L* a* b*) values of ground whole sorghum and sorghum bran

Whole ground sorghums	L*	a*	b*
High tannin sorghum bran	60.3a	11.8d	13.9c
White sorghum	88.2d	2.6a	17.5d
High tannin sorghum	68.6c	7.4b	11.7b
Black sorghum	63.3b	6.2c	7.9a

Data values with different letters are significantly different at $P<0.05$

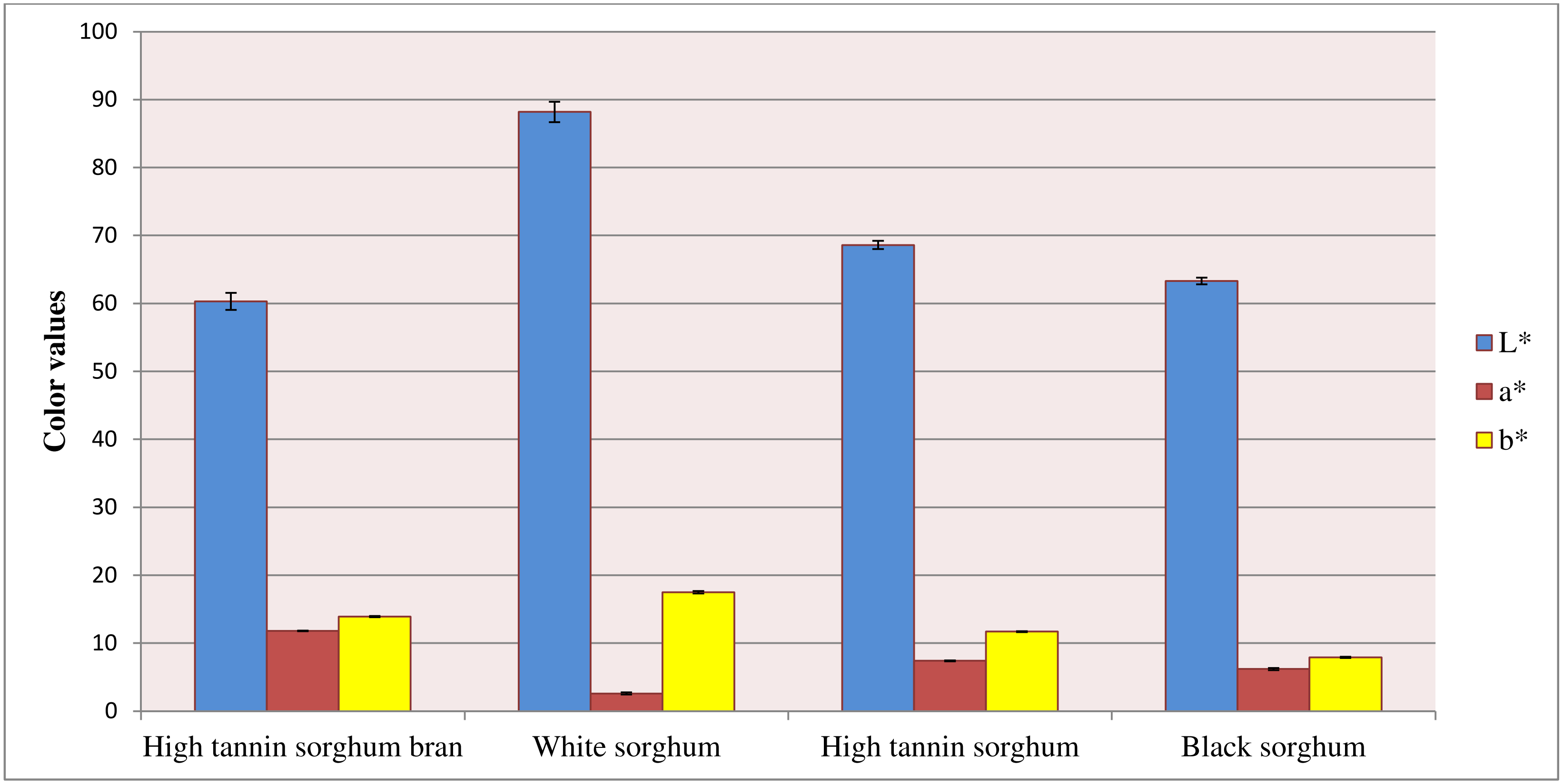


Figure 10: Color (L* a* b*) values of whole ground sorghum and high tannin sorghum bran

4.3 COMPOSITION OF SORGHUM GRAIN

Protein contents of high tannin (11.6±0.35) and black (12±0.12) sorghum were significantly higher than that of white (10.05±0.33) sorghum. High tannin (67.1±1.5) and black sorghum (66.7±0.97) varieties had significantly ($P<0.05$) less total starch than that of white sorghum (78.7±1.7) (Table 13). The starch and protein contents of the sorghum varieties were within the normal ranges reported for sorghum (Rooney & Waniska, 2001). All sorghum varieties were morphologically different with significantly ($P<0.05$) different starch content. The starch content in sorghum has a range of 55.6–75.8% (Serna-Saldivar & Rooney, 1995) and is normally ≈70% on dry weight basis. Sorghum starch granules are normally polygonal and are very similar to corn starch granules. A normal sorghum starch granule contains 23-30% amylose (Waniska & Rooney, 2000). Austin (2008) reported that the dissected white and black sorghum grains showed a higher proportion of hard endosperm while high tannin sorghum had a higher proportion of floury endosperm. The soft endosperm in the tannin sorghum was a reason for lower hardness index compared to white sorghum.

Table 13: Composition of white, high tannin and black sorghum grains

Sorghum Variety	Moisture	Protein	Starch
White (Atx635*RTx436)	10.9ab	10.0a	73.7b
High Tannin(CSC3*R28)	11.3b	11.6b	67.1a
Black (A0530/BlackTX430)	10.5a	12.0b	66.7a

Data values with different letters are significantly different at $P<0.05$

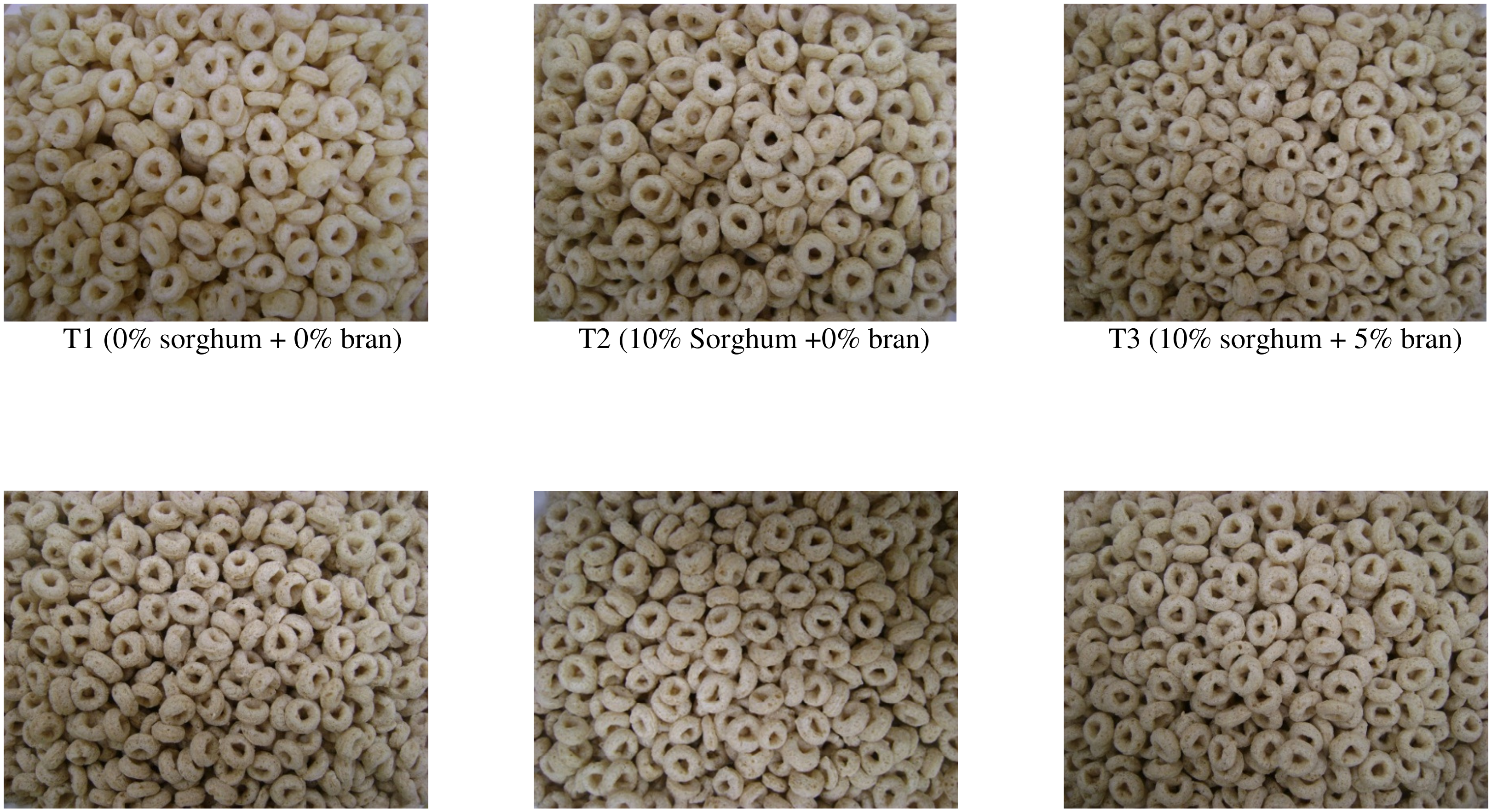
4.4 EXPERIMENT 1: DEVELOPMENT OF GLUTEN CONTAINING RTE BREAKFAST CEREALS BY USING HIGH TANNIN SORGHUM AND BRAN

Whole grain high tannin sorghum and high tannin sorghum bran were hammer milled. Particle size distribution (Table 14) of sorghum bran indicated that the majority was retained over sieve # 60 and sieve # 40. Whole ground high tannin sorghum particles varied from sieve # 40 to sieve # 80 with a maximum retention at sieve # 60. Sorghum bran was difficult to grind therefore it remained coarser, a major portion was retained on sieve # 40 while whole grain high tannin sorghum milled well due to floury endosperm. Particle size greatly affects extrudate characteristics by altering the extent at which starch is modified inside the extruder. Finer particles tend to produce products with softer textures and smaller cell structures, while coarse particle size materials give extrudates with crunchier texture and larger cell structure.

Table 14: Particle size distribution (%) of whole ground high tannin sorghum and high tannin sorghum bran(Experiment 1)

US standard sieve #	Sorghum**	Bran***
40	28	54
60	40	32
80	24	8
Pan	8	5

Over the sieve
**Whole ground high tannin sorghum
***High tannin sorghum bran



T1 (0% sorghum + 0% bran) T2 (10% Sorghum +0% bran) T3 (10% sorghum + 5% bran)

T4 (10% sorghum + 10% bran) T5 (20% sorghum + 0% bran) T6 (20% sorghum +5% bran)

Figure 11: Extrudates with different levels of high tannin whole ground sorghum and high tannin bran (Experiment 1)

T7 (20% sorghum + 10% bran)

T8 (30% sorghum + 0% bran)

T9 (30% sorghum + 5% bran)

T10 (30% sorghum + 10% bran)

T11 (40% sorghum + 0% bran)

T12 (40% sorghum + 5% bran)

Figure 11 Continued.

T13 (40% sorghum + 10% bran)

Figure 11 Continued.

4.4.1 Color of Extrudates

There was a significant (P<0.05) difference in the color of all the extrudates (Table 15). Control (T1) had the highest L* value (83.1±0.6) and there was a continuous reduction in L* value by increasing the sorghum and bran level in the extrudates. This indicated that by increasing the sorghum and bran level the color was going to be darker. As expected, treatment 13 (40% sorghum +10% bran) had the lowest L* value (66.6 ± 0.7) which means it has the darkest color in all treatments. The T8 (30% sorghum + 0% bran) also had a high L* value (83.5 ± 0.8) which was due to contamination of control (T1) in this treatment.

The a* value of all the treatments were significantly (P<0.05) different and positive, which indicated that all the treatments were more red than green. As expected treatment 13 (40% sorghum + 10% bran) had the highest a* value (10.1±0.07) which was not statistically different from treatment 11 (40% sorghum + 0% bran). This indicated that with increased levels of high tannin sorghum, additional high tannin bran does not have a significant effect on color. Control (T1) had the lowest a* value (4± 0.1). There was a significant difference in b* value of treatments. It decreased by increasing the sorghum and bran contents. Control (T1) had the highest b* value (25.3 ± 0.1) indicating that it was more yellow than blue. The b* value of treatment 13 (18.4 ± 0.1) indicated it was less yellow.

Table 15: Properties of extrudates with different levels of whole ground sorghum and high tannin sorghum bran (Experiment 1)

Treatments	Bulk density (g/L)	Expansion ratio	Color L*	Color a*	Color b*
0% sorghum + 0% bran (T1)	61a	6.58ef	83.1h	4.0b	25.3k
10% sorghum + 0% bran (T2)	64a	6.94f	76.4g	6.9c	24.1j
10% sorghum + 5% bran (T3)	73bc	5.93cd	75.7g	7.0c	22.5h
10% sorghum + 10% bran (T4)	70b	5.94cd	73.8f	8.2f	21.3g
20% sorghum + 0% bran (T5)	70b	6.27de	72.8e	7.4d	21.0f
20% sorghum +5% bran (T6)	74c	6.61ef	73.9f	8e	20.7e
20% sorghum + 10% bran (T7)	80de	5.90cd	71.9d	8.4g	19.8c
30% sorghum + 0% bran (T8)	88f	5.70c	83.5h	3.5a	23.8i
30% sorghum + 5% bran (T9)	83e	5.34b	71.3d	8.5g	20.3d
30% sorghum + 10% bran (T10)	79d	5.13ab	69.1c	9.6h	21.0f
40% sorghum + 0% bran (T11)	103h	4.96a	69.9c	10.1i	20.1d
40% sorghum + 5% bran (T12)	97g	4.86a	68.0b	9.7h	18.9b
40% sorghum + 10% bran (T13)	108i	4.93a	66.6a	10.1i	18.4a

Values in each column with different letters are significantly different at $P<0.05$

4.4.2 Bulk Density and Expansion Ratio of Extrudates

In experiment 1 the objective was to get the best shape of product by using different levels of high tannin whole ground sorghum and sorghum bran. To achieve this objective different processing conditions were used (Table 10). The extruder screw speed varied from 308-406 rpm, water flow to extruder barrel was 0.7-4.3 kg/hr and knife speed was 70-90% of maximum.

There was a significant ($P<0.05$) difference in the bulk density (Table 15) of extrudates in experiment 1 (Figure 11). There was a positive correlation ($R^2= 0.7965$) between the bulk density and sorghum and bran level. Overall by increasing the quantity of whole ground high tannin sorghum and sorghum bran the bulk density of extrudates was increased (Figure 12). Bulk density of control, without any sorghum or bran was lowest (61±2.2g/L) of all extrudates and increased steadily with the addition of high tannin sorghum and sorghum bran. In T3, bulk density was 73±0.4 g/L with the addition of 10% whole ground high tannin sorghum and 5% high tannin bran, while it was decreased (70±1g/L) when an additional 5% high tannin bran was added in treatment 4. This variation in bulk density was because of decreased moisture contents. More moisture is required to hydrate the bran particles properly but here less water was available therefore more expansion occurred and bulk density was decreased. Similar results were reported by Hernandez-Diaz et al. (2007). With the addition of 20% sorghum and 0% bran, bulk density was 70±0.9g/L and significantly ($P<0.05$) increased to 74±0.6g/L and 80 g/L when 5 and 10% bran was added respectively. Again moisture contents of flour mix and water addition during extrusion played an important role in bulk density of extrudates.

When 30% sorghum and 0% bran was used, the bulk density was 88±0.3g/L and it was significantly decreased to 79±0.3g/L when 10% bran was added in the flour. Hernandez-Diaz et al. (2007) mentioned that with low moisture contents, bulk density was increased by increasing levels of bran and decreased for higher moisture contents. Maximum bulk density (108±1g/L) was obtained with 40% ground sorghum and 10% bran while 40% ground sorghum without bran gave a bulk density of 103 ±0.4 g/L. Similar results were reported by Hashimoto & Grossmann (2003) in extrudates of cassava bran/cassava starch.

Expansion ratio of extrudates was significantly decreased by increasing the sorghum and bran. It had a negative correlation (R^2=0.80) with the sorghum and bran addition in the composite flour (Figure 12). Expansion ratio of 10% sorghum without additional bran was higher (6.94) than that of control (6.58). Expansion ratio was decreased (5.94) when 10% high tannin sorghum bran was added to composite flour which already contained 10% whole ground sorghum. There was no significant difference in the expansion ratio of extrudates developed by using 10 and 20% whole ground sorghum and 10% high tannin bran. By using 30% sorghum and 10 % bran the expansion ratio of extrudates was 5.13 which was reduced to 4.93 when 40% sorghum and 10% bran was used. Therefore the expansion depends on the concentration of fiber present. Fibers are also able to bind some of the moisture present in the matrix, thus reducing its availability for expansion (Onwulata et al., 2001). Increasing moisture content during extrusion gave less viscous dough which caused shrinking and collapse of the extrudates under the high vapor pressure generated in the extruder (Moraru & Kokini, 2003) thus reducing expansion and increasing bulk density. In experiment 1 gluten containing RTE breakfast cereals were produced with desired round shape. The brightness of the cereals was decreased by increasing the sorghum and bran levels. Whole ground sorghum and bran extrudates had decreased expansion and increased bulk density particularly at increased levels of sorghum and high tannin bran. Increased fiber content to an optimum level reinforces extrudate structure and bulk density. However, by adjusting the levels of bran in the formulation and extrusion parameters, a product with good quality characteristics can be obtained.

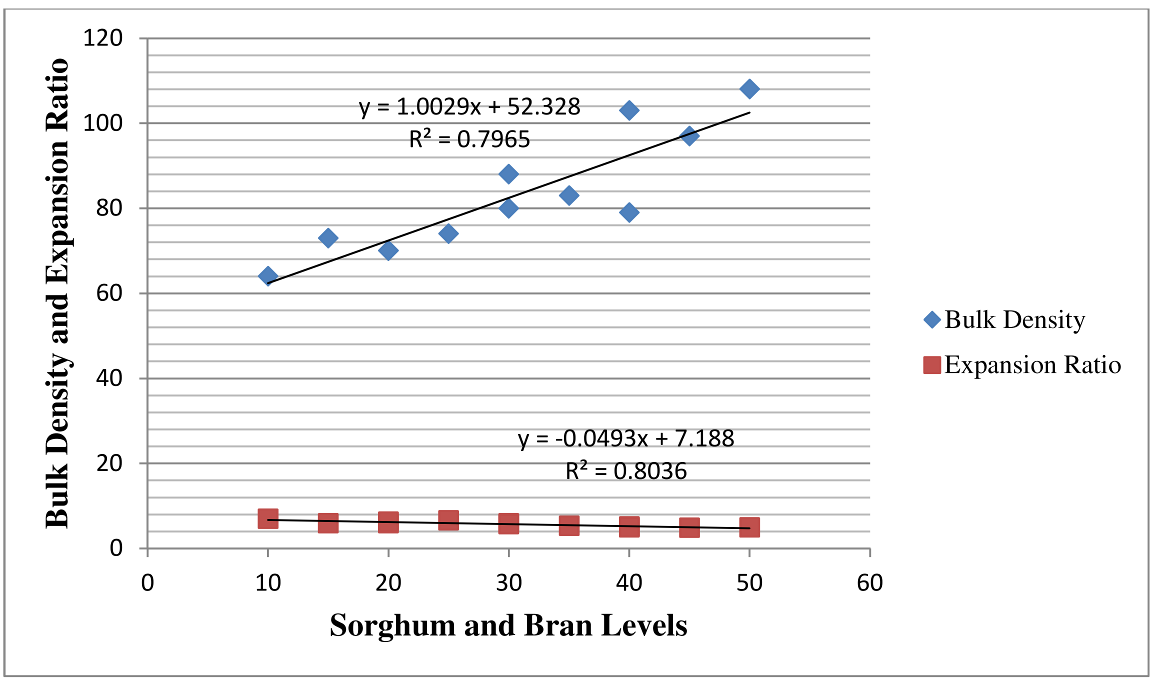


Figure 12: Correlations of sorghum and bran addition with the bulk density and expansion ratio of extrudates (Experiment 1)

4.5 EXPERIMENT 2: DEVELOPMENT OF GLUTEN FREE, RTE BREAKFAST CEREALS AND SNACKS BY USING WHOLE GROUND WHITE SORGHUM AND HIGH TANNIN BRAN

In experiment 2, whole ground white sorghum (Figure 13) with additional high tannin sorghum bran was used to develop breakfast cereals. For this purpose two treatments were designed. In one treatment 50% white sorghum with 5% high tannin bran while second treatment consisted of 60% white sorghum with additional 10% high tannin bran. To increase the protein contents, 3% soy isolates were added to the formulation.

Whole white sorghum

Whole ground white sorghum

Figure 13: White sorghum whole and ground (Experiment 2)

Milling yield of whole white sorghum (Figure 13) was 100% using a hammer mill. High-tannin bran was also hammer milled into smaller particles for better incorporation in the blend and for ease in extrusion. Particle size distribution of whole ground white sorghum and high tannin bran (Table 16) indicated that 58% of sorghum flour passed through sieve # 40, while 45% stayed at # 60. In case of high tannin sorghum bran, 32 % of material passed through sieve # 40 while 54% stayed over a # 60 sieve. High tannin sorghum bran was 30% decorticated fraction of sorghum and it could contain some endosperm portion. Therefore, 14% of high tannin sorghum bran fraction which passed through # 60 sieve could be a part of endosperm it contained.

Table 16: Particle size distribution (%) of whole ground white sorghum and high tannin sorghum bran (Experiment 2)

US standard sieve #	Sorghum**	Bran***
20	0	0
40	42	32
60	45	54
80	3	8
Pan	8	6

Over the sieve
**Whole ground white sorghum
***High tannin sorghum bran

Extrudates with 50% white sorghum and 5 % high tannin bran

Sugar coated extrudates with 50% white sorghum and 5 % high tannin bran

Extrudates with 60% white sorghum and 10 % high tannin bran

Sugar coated extrudates with 60% white sorghum and 10 % high tannin bran

Figure 14: Extrudates with 50-60% whole ground white sorghum and 5-10% high tannin bran (Experiment 2)

Table 17: Attributes of extrudates with different sorghum and bran levels (with and without sugar coating) (Experiment 2)

	Expansion Ratio	Bulk Density (g/L)	Bowl life (min)	Hardness*	WSI	WAI	Antioxidant activity (µmol TE/g)
50% Sorghum + 5% Bran	3.38a	130a	11.4b	26.6a	0.103a	7.0b	20a
50% Sorghum + 5% Bran (Sugar coated)	-	150a	10.0a	23.8a	0.191c	7.0b	-
60% Sorghum + 10% Bran	2.95b	170b	18.4c	23.7a	0.098a	6.2a	49b
60% Sorghum + 10% Bran (Sugar coated)	-	180b	17.6c	26.6a	0.159b	6.2a	-

Values in each column with different letters are significantly different at P<0.05
*Maximum compression force (N)

4.5.1 Expansion Ratio of Extrudates

Both types of extrudates (Figure 14) had a significant (P<0.05) difference in expansion ratio (Table 17, Figure 15). Extrudates with 50% sorghum and 5% bran were more expanded (3.38±0.075) than the extrudates which had 60% sorghum and 10% bran (2.95±0.036). Extrudates made with less bran and sorghum expanded more and in general had more expansion ratio and lowest bulk density. High bran contents and use of more whole sorghum restricted the expansion of extrudates. The expansion ratio of extrudates decreased with increasing fiber content (Rinaldi et al., 2000; Acosta-Sanchez, 2003). The effect of fiber on extrudate expansion is concentration-dependent, regardless of the source of fiber (Hernandiz-Diaz, 2007).

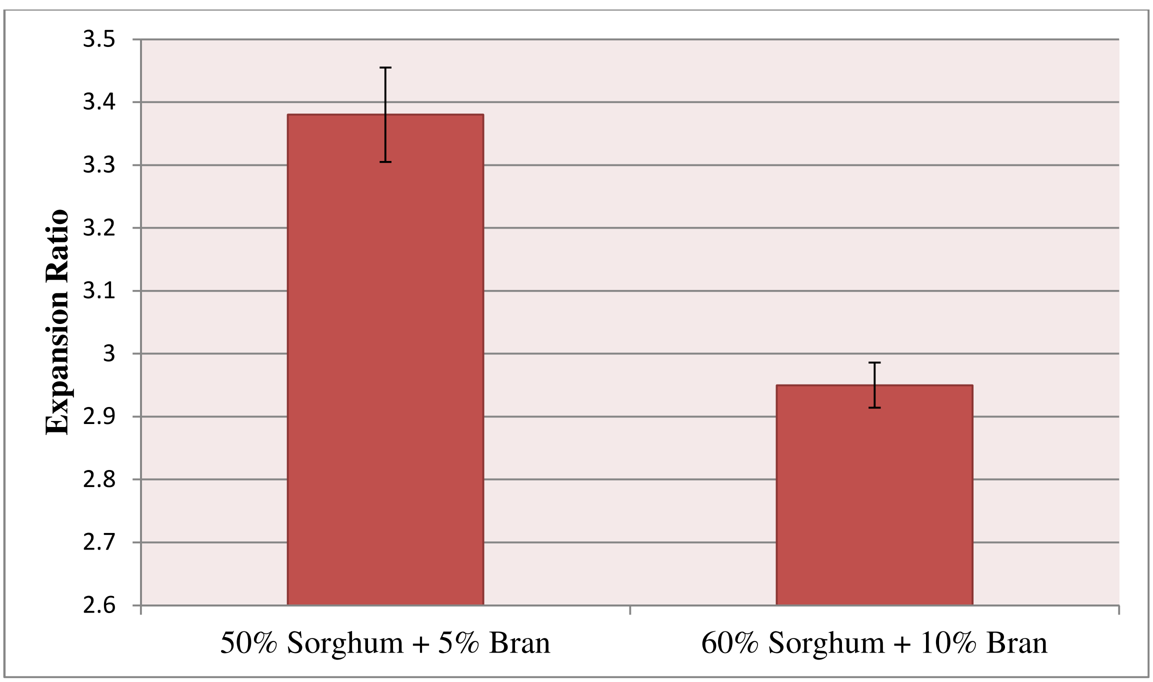


Figure 15: Expansion ratio of extrudates with different sorghum and bran levels (Experiment 2)

4.5.2 Bulk Density of Extrudates

Bulk density and expansion ratio are important factors for extrudates because they are associated with product crispness, water absorption, water solubility and crunchiness (Ali et al., 1996). Bulk density of extrudates made with 50% whole ground white sorghum and 50% bran was significantly ($P<0.05$) lower (128g/L) than the bulk density of extrudates made with 60% whole ground white sorghum and 10% bran (170g/L) (Table 17). The same difference was observed when both types of extrudates were coated with sugar (Figure 16). Extrudates made with whole sorghum grain without any decortication had more bulk density than the extrudates made with 20% decortication (Acosta-Sanchez, 2003). Presence of fiber particles tended to rupture the cell walls before the gas bubbles had expanded to their full potential (Lue et al., 1991).

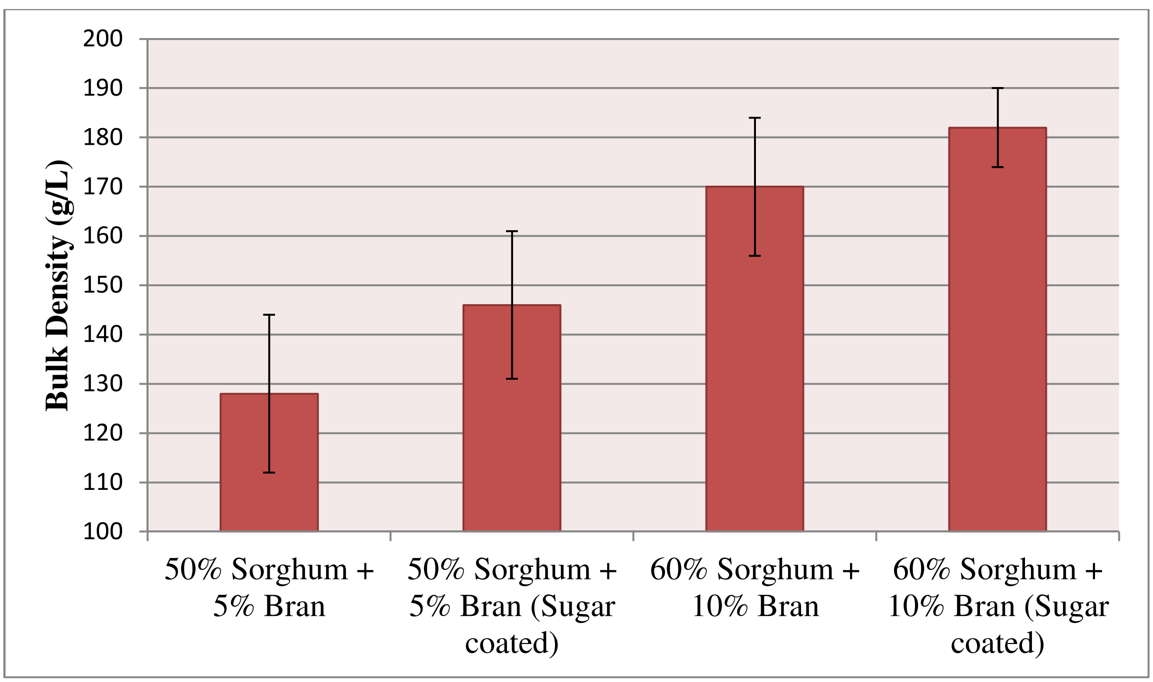


Figure 16: Bulk density of extrudates (with and without sugar coating) (Experiment 2)

4.5.3 Bowl Life of Extrudates

Bowl life of extrudates (Figure 17) made with 50% sorghum and 5% bran was significantly lower (11.4±1.1 min) than that of extrudates made with 60% sorghum and 10% bran (18.4±0.55 min). The bowl life of extrudates made with 50% sorghum and 5% bran is lower than that of other cereals but overall this is a fairly good bowl life. Coating of extrudates with sugar solution reduced the bowl life. In the case of cereals containing 50% sorghum and 5% bran, bowl life reduction was significant (Table 17). This reduction could be due to the abrasive action which took place during coating of cereals, damaging the outer layer of gelatinized starch which was acting as a barrier to milk absorption and swelling in the cereals.

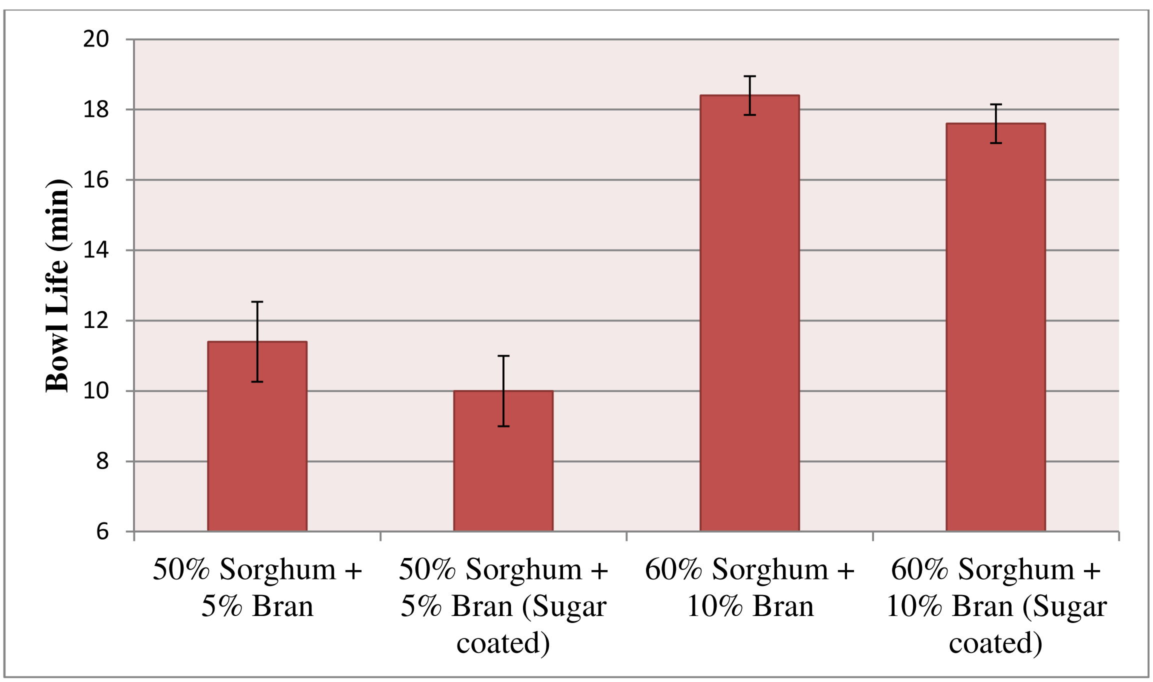


Figure 17: Bowl life of extrudates (with and without sugar coating) (Experiment 2)

4.5.4 Hardness (Maximum Compression Force) of Extrudates

The force required to completely disintegrate the extrudates is called maximum compression force (N) and it indicates the hardness of extrudates. Maximum compression force (N) required to break the extrudates (Table 17, Figure 18) made with 50-60% sorghum and 5-10% bran varied between 23.7-26.6 N but this difference was not significant ($P<0.05$). Fibers increased hardness and decreased porosity (Yanniotis et al., 2007) but in this experiment the difference in hardness was not significant. A possible reason of this non-significant difference could be the increased temperature that would decrease melt viscosity, but it also increased the vapor pressure of water (Ding et al., 2005). This favored the bubble growth which was the driving force for expansion that produces low density soft extrudates (Ding et al., 2005).

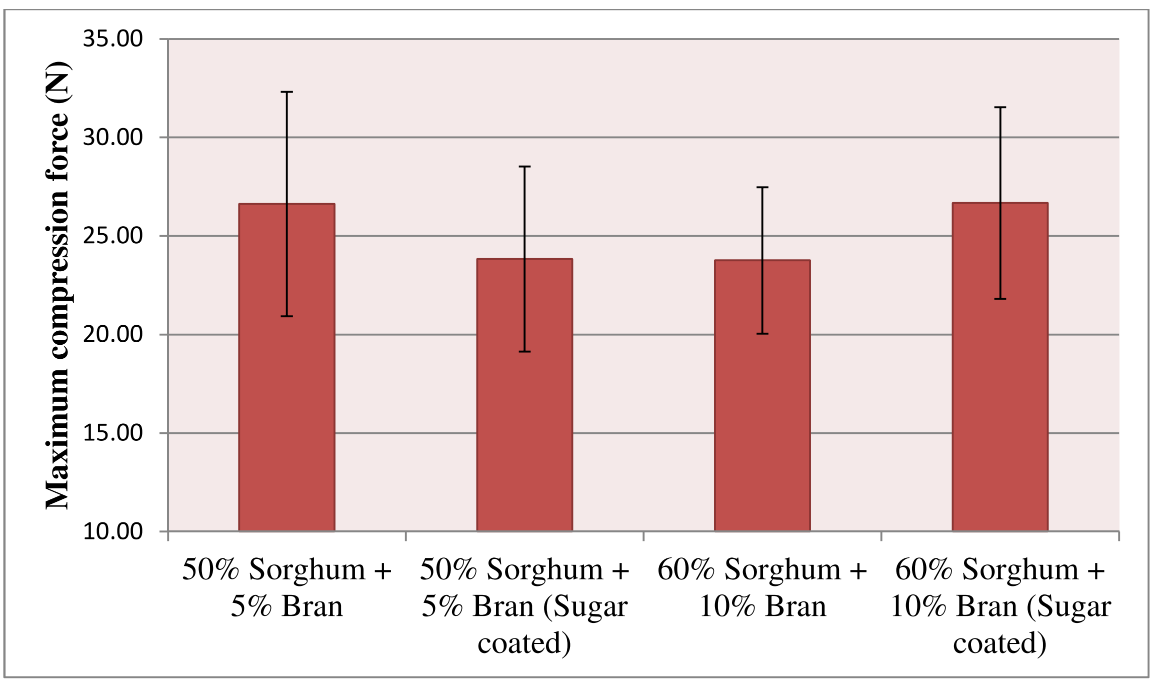


Figure 18: Hardness of extrudates (with and without sugar coating) (Experiment 2)

4.5.5 Water Soluble Index (WSI) of Extrudates

The WSI has been used as a measure for starch degradation in extrudates. At lower WSI there is minor degradation of starch which lowers soluble molecules in the extrudates. There was a non-significant difference (P<0.05) in WSI of both types of extrudates made with different levels of sorghum and bran (Figure 19). During extrusion the processing conditions were the same therefore, formulation itself had a non-significant effect on the WSI. After coating the WSI of both types of extrudates increased because water added in the sugar solution hydrated the extrudates starch and made it more soluble. Another cause of increased solubility in sugar coated cereal could be the sugar which increased the soluble contents of the extrudates. The increased feed rate at low moisture would decrease WSI of extrudates (Ding et al., 2005).

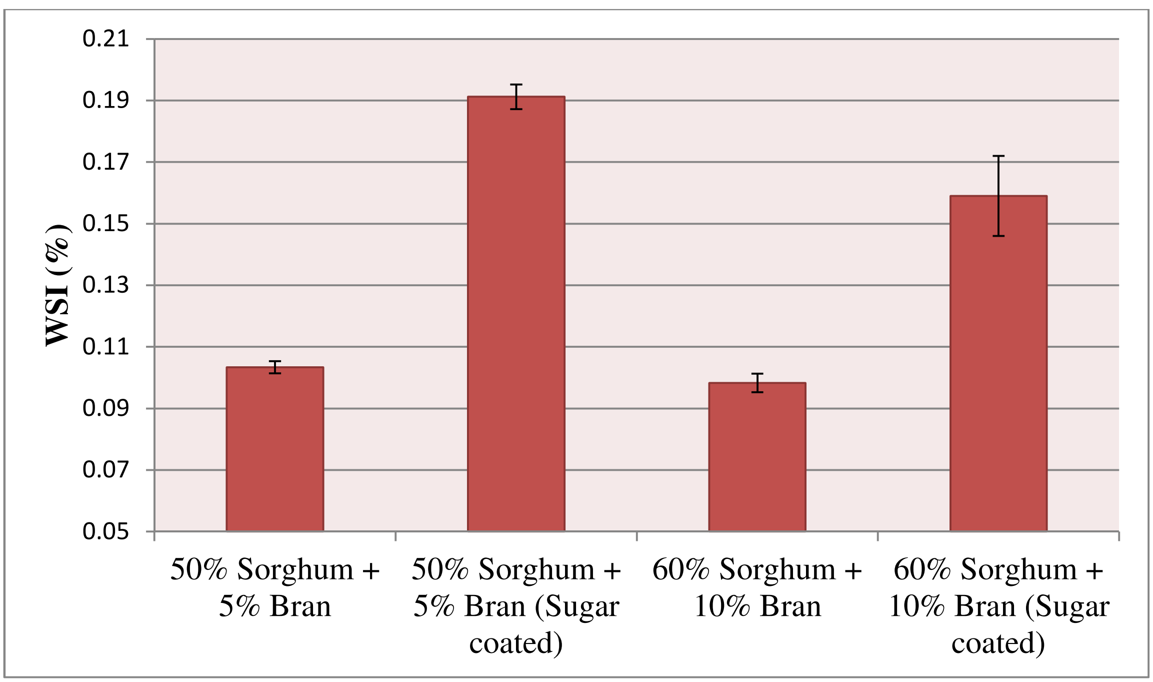


Figure 19: Water soluble index of extrudates (Experiment 2)

4.5.6 Water Absorption Index (WAI) of Extrudates

The WAI is an indicator of the functional properties of starch present in extrudates and the stability of starch–bran composites against water. According to Mason & Hoseney (1986), the WAI measures the volume occupied by the starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion. The WAI of extrudates made with 60% sorghum and 10% bran was significantly ($P<0.05$) lower (6.23±0.05g/g) than that of extrudates made with 50% sorghum and 5% bran (7.0±0.24g/g) (Figure 20). Starch-bran complexes prevent the leaching of amylose and decrease the water uptake and swelling, which could also reduce the solubility of the starch too.

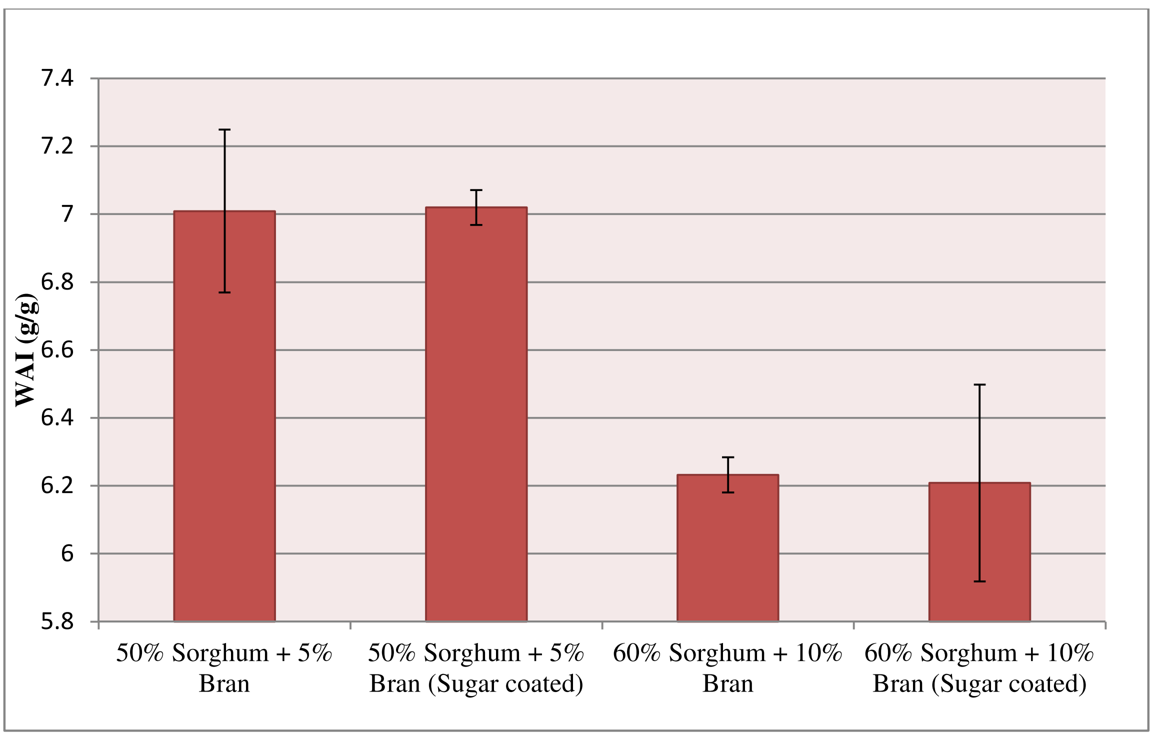


Figure 20: Water absorption index of extrudates (Experiment 2)

4.5.7 Antioxidant Activity of Extrudates

Antioxidant activity of extrudates was significantly ($P<0.05$) different in both types of extrudates. In the case of 60% sorghum and 10% high tannin bran antioxidant activity of extrudates was higher (49±5 µmolTE/g) than that of the 50% sorghum and 5% bran (20±3 µmolTE/g) (Figure 21). Extrusion cooking significantly reduced measurable antioxidant activity, when compared to that of the unprocessed grain (Awika, 2003). In this experiment we did not analyze the antioxidant activity of flour mix before extrusion therefore, we were unable to identify that how much antioxidant activity were retained after extrusion.

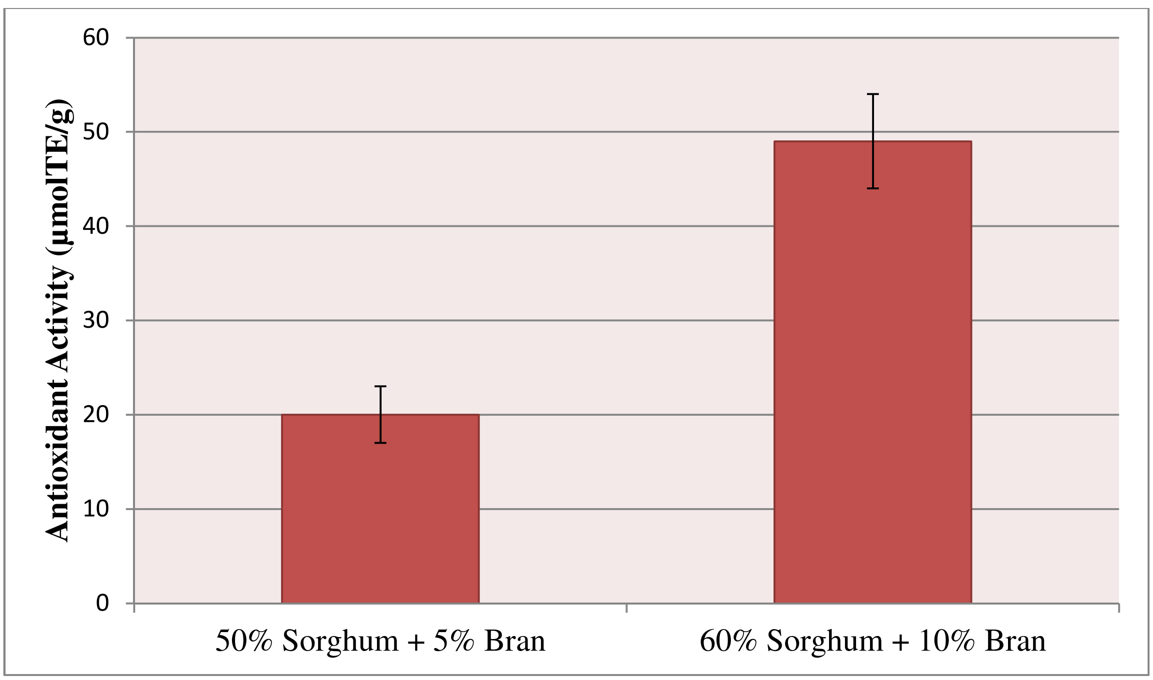


Figure 21: Antioxidant activity of extrudates (Experiment 2)

4.5.8 Conclusion (Experiment 2)

Whole ground white sorghum (50-60%) and high tannin bran (5-10%) were extruded to develop breakfast cereal. Bulk density and expansion ratio of extrudates were affected by the addition of whole ground sorghum and high tannin bran. Expansion ratio was reduced for 60% sorghum and 10% bran, and as expected bulk density was increased. Bowl life was increased to 18 min with 60% sorghum and 10% bran. There was no difference in hardness of extrudates but WAI in 60% sorghum and 10% bran was significantly less than that of 50% sorghum and 5% bran. Antioxidant activity was significantly different in both extrudates and varied from 20-49 µmolTE/g.

4.6 EXPERIMENT 3: DEVELOPMENT OF GLUTEN FREE RTE BREAKFAST CEREALS AND SNACKS BY USING WHITE, HIGH TANNIN AND BLACK SORGHUM

The third experiment was designed to use 80% of white, black and high tannin whole ground sorghum (Figure 8) with 20% of other ingredients (Table 18).

Major proportion (55%) of high tannin whole ground sorghum stayed on sieve # 40, which was more than the black (39%) and white (42%) sorghum. White sorghum with high percentages of corneous endosperm, had pericarps that more cleanly separated from intact endosperm. Black sorghum with floury endosperm, however, tends to give a higher percentage of fine endosperm particles mixed with the bran fraction.

Table 18: Particle size distribution (%) of white, high tannin and black whole ground sorghum (Experiment 3)

US standard sieve #	White sorghum	High tannin sorghum	Black sorghum
20	1a	0a	0a
40	42d	55d	39c
60	46e	38c	48d
80	3b	4b	6b
Pan	8c	3b	5b

Values in each column with different letters are significantly different at $P<0.05$ over the sieve

Product obtained (extrudates, Figure 22) from experiment 3 were coated with sugar (Figure 23) and with cheese (Figure 24) by using the method described in materials and methods.

80% white sorghum extrudate | 80% high tannin sorghum extrudate | 80% black sorghum extrudate

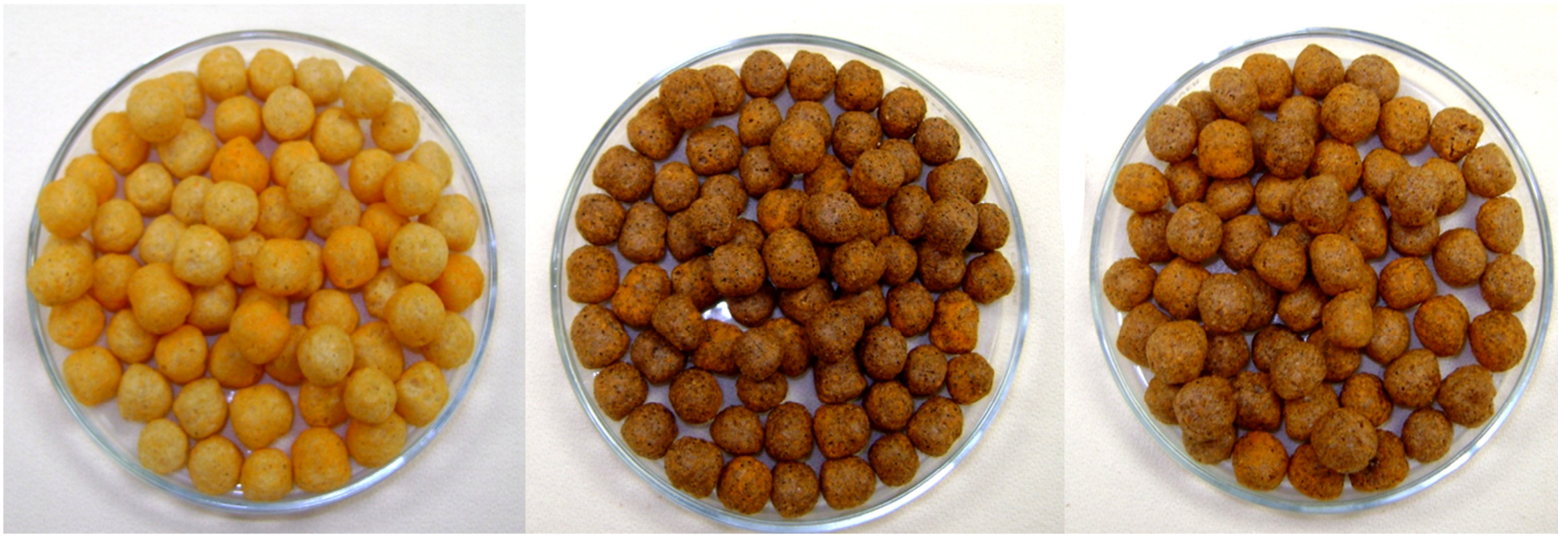
Figure 22: Uncoated extrudates from different types of whole ground sorghum (Experiment 3)

80% white sorghum extrudate after sugar coating

80% high-tannin sorghum extrudate after sugar coating

80% black sorghum extrudate after sugar coating

Figure 23: Sugar coated extrudates from different types of whole ground sorghum (Experiment 3)



80% white sorghum extrudate after cheese coating

80% high tannin sorghum extrudate after cheese coating

80% black sorghum extrudate after cheese coating

Figure 24: Cheese coated extrudates from different types of whole ground sorghum (Experiment 3)

Table 19: Attributes of extrudates from different types of sorghum (80%) (Experiment 3)

Extrudates	Expansion ratio	Bulk density (g/L)	Bowl life (min)	Hardness*	Antioxidant activity µmolTE/g		
					Before ext.	After ext.	% of total retained
Yellow corn flour	3.64b	66c	2.6a	ND	ND	ND	ND
White sorghum	3.86d	58a	3.3a	45.2b	22a	31.7a	144
High tannin sorghum	3.78c	58a	3.6a	53.1c	307c	224c	73
Black sorghum	3.41a	60ab	3.0a	42.9a	174b	148b	85

Values in each column with different letters are significantly different at $P<0.05$
*Maximum compression force (N)
ND: No data
ext: Extrusion

4.6.1 Expansion Ratio of Extrudates

There was a significant (P<0.05) difference in the expansion ratio of all extrudates. The expansion ratio of black sorghum extrudates (3.41±0.0) was lower than that of white (3.86±0.0) and high-tannin (3.78±0.0) sorghum extrudates (Table 19, Figure 25). The expansion ratio of corn extrudates (3.64±0.01) fell in between the sorghum extrudates. Fibers are able to bind some of the moisture present in the matrix, thus reducing its availability for expansion (Onwulata et al., 2001). Increasing moisture content during extrusion produce less viscous dough which causes shrinking and collapse of the extrudates under the high vapor pressure generated in the extruder (Moraru & Kokini, 2003) thus reducing expansion. In the case of corn flour extrudates, there was a lack of strength in the walls of extrudates therefore the walls collapsed after expansion.

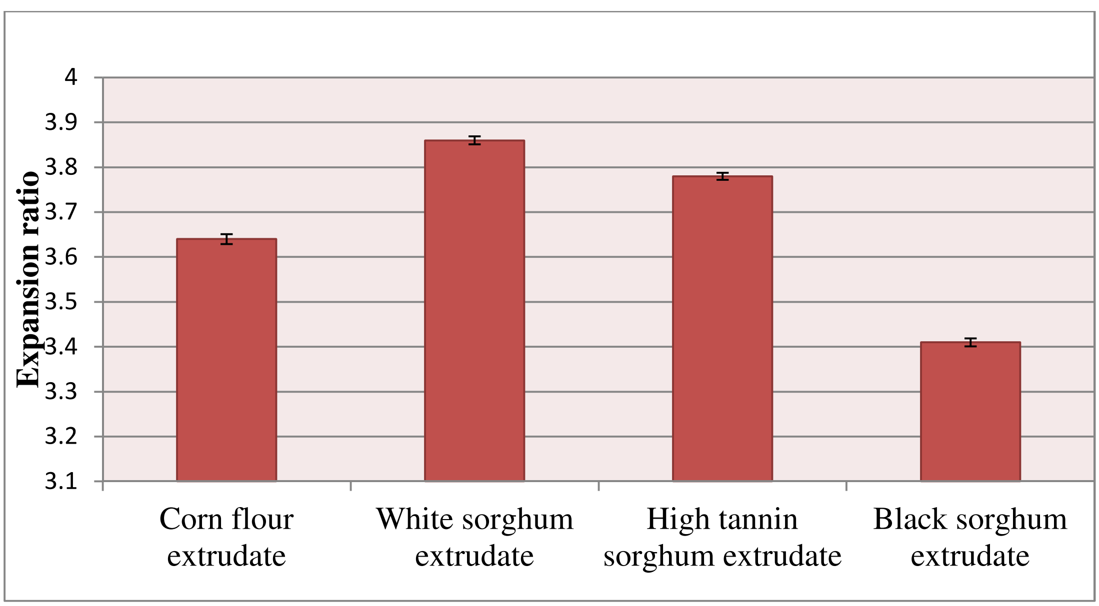


Figure 25: Expansion ratio of extrudates (Experiment 3)

4.6.2 Bulk Density of Extrudates

There was a significant (P<0.05) difference in the bulk density of corn and sorghum extrudates (Table 19) developed in experiment 3. Black sorghum extrudates had the highest bulk density (60±1.3g/L), white and high-tannin sorghum extrudates were similar in bulk densities (Figure 26). The corn flour produced extrudates with larger air cells but because of thin walls it collapsed and had more bulk density than the sorghum extrudates. Black sorghum contained thick bran which reduced expansion by rupturing the cell wall at pre-expanded stage and thus increased the bulk density of extrudates. The extrudate density was most dependent on feed moisture and temperature. Increased feed moisture leads to a sharp increase in extrudate density however increased barrel temperature caused a slight decrease in density (Ding et al., 2005).

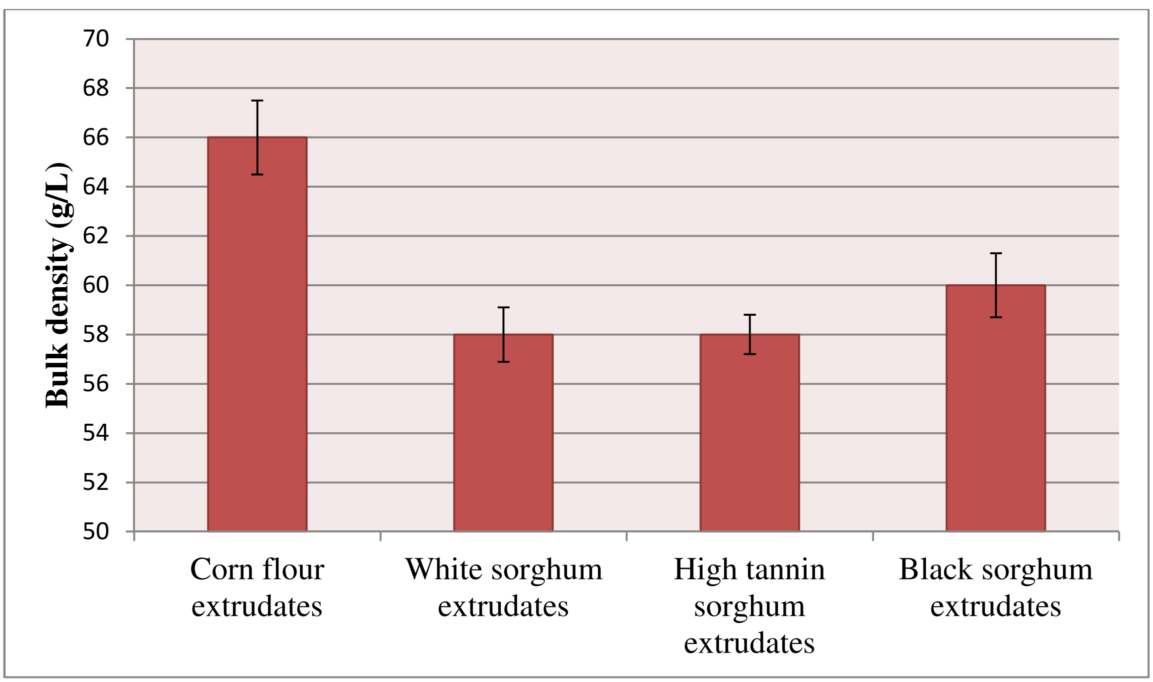


Figure 26: Bulk density of extrudates (Experiment 3)

4.6.3 Bowl Life of Extrudates

The bowl life of extrudates made with corn and different sorghums varied from 2.6 to 3.6 min (Figure 27). Bowl life is related to the starch degradation and it could be enhanced by using high moisture contents during extrusion. In experiment 2, water used was 4.1 kg/hr and bowl life was up to 18 min for 60% sorghum + 10% bran extrudate while in experiment 3 water flow to the extruder was 1.1 kg/hr only and bowl life was reduced to 3.6 min. These results showed that during extrusion with additional bran and at higher moisture contents, there is more degradation of starch which reduced the water uptake of extrudates and ultimately increased the bowl life.

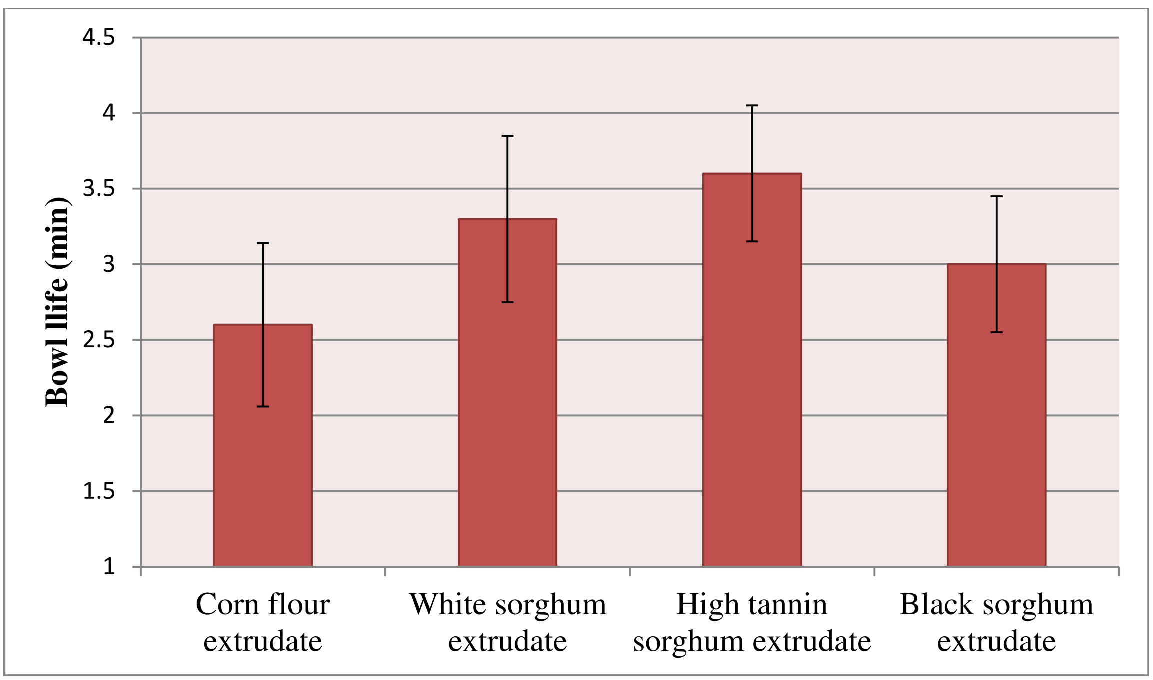


Figure 27: Bowl life (min) of extrudates (without sugar coating) made with different types of sorghum and corn (Experiment 3)

4.6.4 Hardness (Maximum Compression Force) of Extrudates

The breaking force (Table 19) used to rupture high-tannin sorghum extrudates (53.1±5.9 N) was higher than the force needed to break white sorghum (45.2±5.4 N) and black sorghum extrudates (42.9±4.3 N). Black sorghum extrudates were softest of all three extrudates and required less force to rupture and break (Figure 28). The floury endosperm of black sorghum could be a contributing factor to the softness. Ding et al. (2005) reported that increasing temperature would decrease melt viscosity, but it also increases the vapor pressure of water. This favors bubble growth which is the driving force for expansion that produces low density products thus decreasing hardness of extrudates.

High-tannin sorghum extrudates were significantly ($P<0.05$) harder to break than the others. In high tannin sorghum the ratio of bran was higher than the endosperm compared to other sorghum types. The particle size distribution of high tannin sorghum (Table 18) indicated that 55% of whole ground sorghum retained at # 40 sieve, which is higher than that of black (39%) and white (42%) whole ground sorghum. Acosta-Sanchez (2003) and Desrumaux et al. (1998) mentioned that extrudates made from raw material with larger particle size had larger air cells and thicker cell walls. Therefore, high tannin sorghum produced extrudates with thicker cell walls, caused by more fiber present in the pericarp of high tannin sorghum compared to white and black sorghum. Ultimately more force was required to break the high tannin extrudates than the white and black sorghum extrudates.

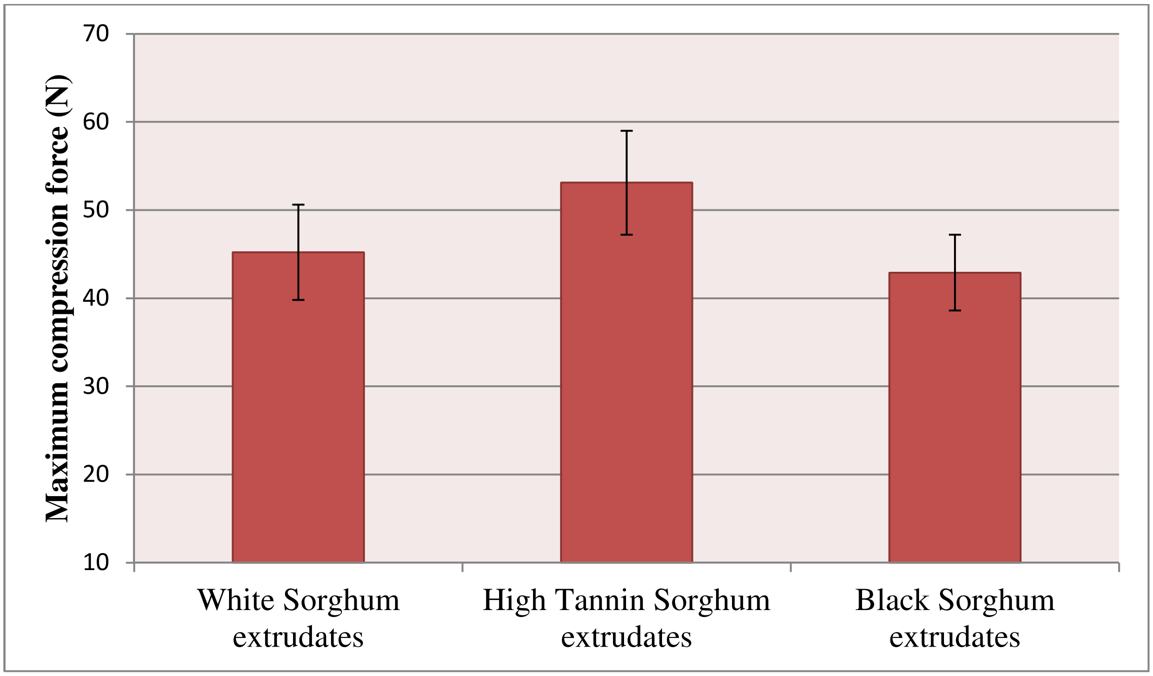


Figure 28: Hardness of extrudates made with different types of sorghum (Experiment 3)

4.6.5 Antioxidant Activity of Extrudates

All three types of sorghum extrudates had significant ($P<0.05$) antioxidant activity after extrusion (Figure 29.). Maximum antioxidant activity of 307±21µmolTE/g was measured in the high-tannin sorghum before extrusion, after extrusion it was reduced to 224±36µmolTE/g, therefore the retention of antioxidant activity was 73%. The difference in antioxidant activity of white and black sorghum, before and after extrusion was not significant (Table 19). The extruded white and black sorghum had 144 and 85% retention of antioxidant activity after extrusion respectively. Increase in the antioxidant activity of white sorghum extrudates is caused by phenolics in the cell wall being solubilized and thus available for analysis. The cleavage and re-association of phenols could occur as well. The antioxidant activity in the extrudates depends largely on the antioxidant activities of the raw material.

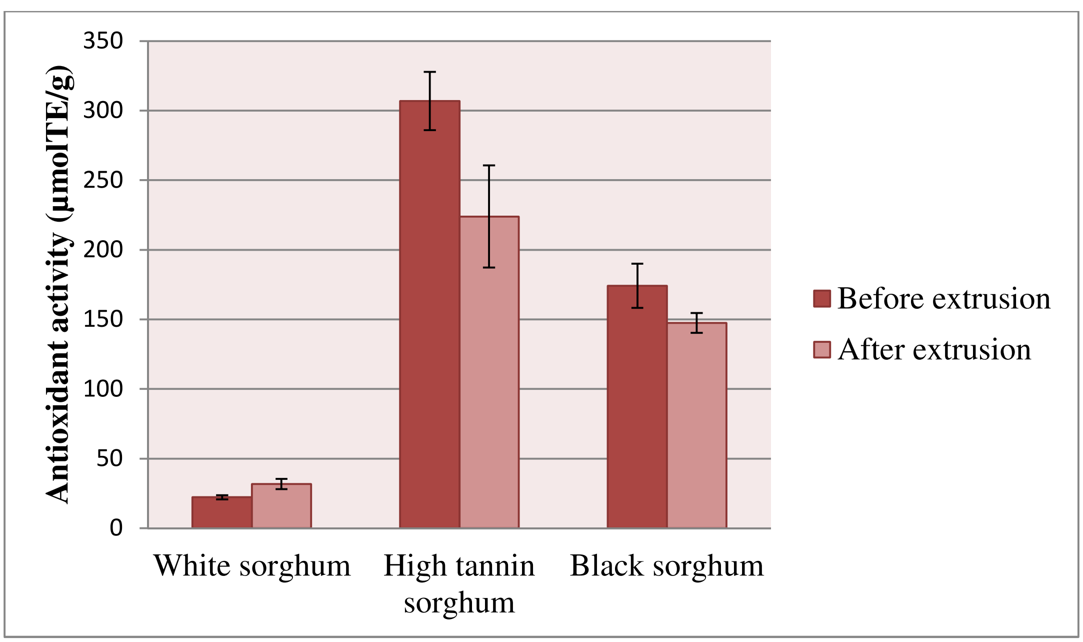


Figure 29: Antioxidant activity of extrudates before and after extrusion (Experiment 3)

4.6.6 Conclusion (Experiment 3)

All three types of sorghum (white, black and high tannin) can be used up to 80% of total formulation weight for the development of breakfast cereal and snacks. Extrudates with 50 and 60% white sorghum and 5-10% high tannin bran had a bowl life up to 18 min however this was reduced to 3 min when 80% sorghum was used without additional bran. Increased bran contents decreased the porosity (Yanniotis et al., 2007) which helped to increase the bowl life of extrudate. Extrudates with 50-60% whole ground white sorghum and 5-10% bran had a higher bulk density with a lower expansion ratio compared to the extrudates made with 80% sorghum. Antioxidant activity of white sorghum extrudates with 5-10% bran was quite low compared to the antioxidant activity of extrudates with 80% sorghum. Color of high tannin and black sorghum extrudates was naturally caramel or chocolate and it could reduce the use of certified food colors in sorghum based cocoa puffs type breakfast cereals. Although extrusion has a detrimental effect on the antioxidant activity of extrudates, the quantity left in the 80% high tannin and black sorghum extrudates was 73 and 85% respectively. While in case of white sorghum extrudates the antioxidant activity was increased up to 144% because of solublization of phenolics present in the cell wall of sorghum which made them available for analysis. In this experiment we used very harsh extrusion conditions, therefore we can conclude that these conditions made phenols available for antioxidant assay.

4.7 EXPERIMENT 4: DEVELOPMENT OF GLUTEN FREE RTE BREAFAST CEREALS AND SNACKS BY USING WHITE, HIGH TANNIN AND BLACK SORGHUM AND HIGH TANNIN SORGHUM BRAN

In the fourth and final experiment all three types of whole ground sorghum up to 85% of total formulation weight with a 0-6% addition of high-tannin sorghum bran were used to develop gluten free breakfast cereals and snacks. The purpose of increasing the sorghum level was to increase the antioxidant activity of final products. In addition high tannin bran incorporation in the formulation increased the bowl life of extrudates as indicated by previous experiments. It also altered the texture by binding with starch during extrusion. High tannin sorghum bran increased the antioxidant activity of breakfast cereal and snacks as the retention of antioxidant depends upon the antioxidant contents of raw material. However bran reduced the expansion of the extrudates and increases bulk density. It was also noticed that high tannin and black sorghum extrudates in experiment 3 had a very desirable natural chocolate or caramel color. All types of whole ground sorghum in combination with different levels of high tannin bran worked very well in the extruder. In this experiment, extrusion conditions were gentle compared to the third experiment. The screw speed was reduced from 411 to 310 rpm and water flow to extruder barrel was increased from 1.1kg/hr to 4.1kg/hr. Reduced rpm of extruder screw allowed more retention time of melt in extruder barrel therefore more disintegration of starch and bran particles took place but excess water makes it gentle. Extrudates from all treatments were highly expanded and had characteristic breakfast cereals and snack appearance (Figures 30-32).

4.7.1 Particle Size Distribution of Whole Ground Sorghums and Bran

Particle size distribution of all types of whole ground sorghum was significantly ($P<0.05$) different. Grain hardness and kernel size affected the particle size distribution of ground sorghum (Table 20). For high tannin bran, more particles (55±2%) stayed on # 40 sieve because the grain was small and contained less endosperm and more bran which was difficult to grind. For white and black whole ground sorghum, 42% and 39% remained over a #40 sieve respectively, while high tannin sorghum bran was 68±1%. In previous experiments high tannin sorghum bran was milled with a hammer mill. In this experiment a Pulverizer (Model H-22, Reynolds engineering and equipment, Inc. Muscatine, IA) was used to grind the high tannin bran which increased the proportion of finer particles as 32% of bran passed through the # 40 sieve.

Table 20: Particle size distribution (%) of sorghum and bran (Experiment 4)

US sieve #	White sorghum*	High tannin sorghum*	Black sorghum*	Bran**
20	0a	0a	0a	0a
40	42d	55d	39c	68d
60	46e	38c	48d	29c
80	3b	4b	6b	3b
Pan	8c	3b	5b	0a

Values in each column with different letters are significantly different at $P<0.05$
*Whole ground
**High tannin sorghum bran

85% white sorghum without additional bran

85% white sorghum with 2% high tannin bran

85% white sorghum with 4% high tannin bran

85% white sorghum with 6% high tannin bran

Figure 30: Whole ground white sorghum extrudates with and without high tannin bran (Experiment 4)

85% high tannin sorghum without additional bran

85% high tannin sorghum with 2% high tannin bran

85% high tannins sorghum with 4% high tannin bran

85% high tannin sorghum with 6% high tannin bran

Figure 31: Whole ground high tannin sorghum extrudates with and without high tannin bran (Experiment 4)

85% black sorghum without additional bran

85% black sorghum with 2% high tannin bran

85% black sorghum with 4% high tannin bran

85% black sorghum with 6% high tannin bran

Figure 32: Whole ground black sorghum extrudates with and without high tannin sorghum bran (Experiment 4)

4.7.2 Color of Extrudates

There was a significant difference in the L* values of all extrudates (Table 21). White sorghum extrudates without additional bran had the highest L* value (80.6±2.2) after the L* value of yellow corn extrudates (99.8±0.37). Extrudates of ground whole white sorghum with different levels of high tannin bran had significant difference ($P<0.05$) in L* values as addition of high tannin bran affected the color of extrudates. There was no significant difference in L* values of high tannin sorghum extrudates except those which had 6% high tannin bran (52.5±0.4).

All samples had positive a* values except corn extrudates (-1.73±0.07), which means all were more red than green except corn extrudates (Figure 33). Black sorghum extrudates with 4 and 6% high tannin bran had the highest a* value indicating that they had maximum red color.

The b* values of extrudates were significantly different within the same type of sorghum. The highest b* values (25.0±0.9) were the white sorghum extrudates without any bran while the lowest b* value was (11.15±0.14) for black sorghum with 2% additional high tannin bran. Thus the addition of high tannin bran affected the whiteness of extrudates as white sorghum and yellow corn extrudates without any bran had the highest b* values. These results agree with the results of Austin (2008). Color of extrudates obtained by using with high tannin sorghum, sorghum bran and black sorghum varied from light brown to dark chocolate color. Due to this unique property of sorghum it can be used as a cocoa replacer in cocoa puff types of cereals.

Table 21: Color values of extrudates made by using different types of sorghum with different levels of high tannin bran

Extrudates	High tannin bran %	L*	a*	b*
White sorghum	0	80.6h	4.86b	25.02j
White sorghum	2	73.1g	7.90c	22.58h
White sorghum	4	70.9f	9.50d	21.14g
White sorghum	6	64.8e	10.85e	20.24f
High tannin sorghum	0	49.5c	11.10ef	13.36c
High tannin sorghum	2	50.2c	11.67f	13.85cd
High tannin sorghum	4	49.8c	12.32h	14.41d
High tannin sorghum	6	52.5d	11.99g	15.33e
Black sorghum	0	46.7b	12.95ij	11.67ab
Black sorghum	2	42.2a	12.67i	11.15a
Black sorghum	4	45.5b	13.33k	11.88ab
Black sorghum	6	45.6b	13.15jk	12.10b
Yellow corn	0	99.8i	-1.73a	23.96i

Values in each column with different letters are significantly different at $P<0.05$

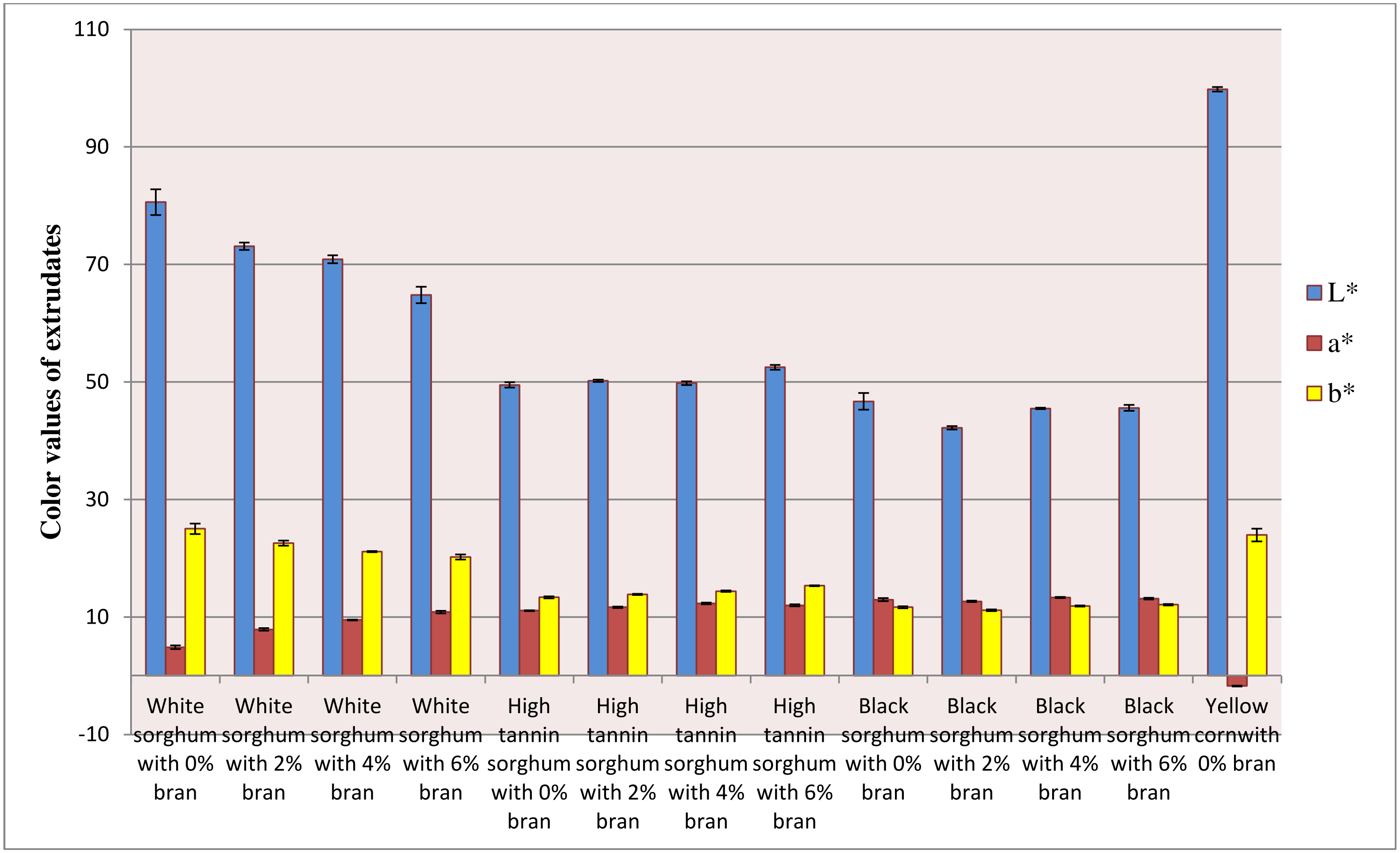


Figure 33: Color values of extrudates with different types of sorghum, with and without high tannin bran (Experiment 4)

Table 22: Attributes of extrudates in experiment 4

Extrudates	Bran *(%)	Bulk Density (g/L)	Expansion Ratio	Max. Compression Force (N)	Bowl Life (min)	WSI(%)	WAI (g/g)
White sorghum	0	56.8b	4.5i	16.5abc	5.3a	52d	2.5a
White Sorghum	2	67.7d	3.8g	16.8bc	8b	ND	ND
White Sorghum	4	72.8e	3.5f	16.1abc	9c	ND	ND
White Sorghum	6	91.2ij	3.3de	14.0ab	8.7bc	20.6a	5.9c
High Tannin Sorghum	0	65.1c	3.4de	14.7ab	10d	31.9c	4.7b
High Tannin Sorghum	2	75.2f	3.3de	15.4abc	11ef	ND	ND
High Tannin Sorghum	4	83.5h	3.2cd	14.1ab	14g	ND	ND
High Tannin Sorghum	6	92.4j	2.8ab	13.9ab	10.3de	20.8a	5b
Black Sorghum	0	75.7f	3.0bc	14.7ab	11.0f	25.8ab	5.2b
Black Sorghum	2	78.6g	2.8b	15.7abc	10.7def	ND	ND
Black Sorghum	4	77.9g	2.8ab	15.8abc	12.7g	ND	ND
Black Sorghum	6	90.4i	2.5a	18.3cd	10.7def	20.8a	4.9b
Yellow corn extrudates	0	42.5a	4.2h	19.5d	5.7a	36.6c	4.8b

Values in each column with different letters are significantly different at $P<0.05$

*high tannin sorghum bran (30% decortication)

4.7.3 Bulk Density and Expansion Ratio of Extrudates

The bulk density of extrudates is a very important product quality attribute because most extruded products are filled by weight and not by volume. Bulk density (Table 22) was increased significantly ($P<0.05$) by increasing the bran content (Figure 34). High tannin sorghum extrudates with additional 6% high tannin bran had maximum bulk density (92.4±1.47) while white sorghum extrudates without bran had low bulk density (56.8±0.86). Increasing the bran in all three types of whole ground sorghum increased the bulk density. Similar results were attained by Turner (2004) when he developed snacks using whole sorghum. All sorghum extrudates had higher bulk densities than the corn flour extrudates. The composition of corn flour is predominately starch, which produces extrudates with a larger network of air cells that are very light. On the other hand whole ground sorghum contained lipids and fiber with less starch which increased the bulk density of sorghum extrudates.

The expansion ratio of extrudates is also related to product quality and is associated with product crispiness, water absorption, water solubility and crunchiness (Ali et al., 1996). Expansion ratio (Figure 35) of extrudates increased as bulk density decreased. Extrudates made without bran or with lower bran levels had better expansion. White sorghum extrudates without bran had more expansion (4.5±0.15) than the corn flour extrudates (4.2±0.57). Reduced expansion due to the presence of bran was also described by Faubion & Hoseney (1982), Grenus et al., (1993) and Acosta-Sanchez, (2003). The expansion ratio of the extrudates decreased with increasing fiber content (Berglund et al., 1994; Rinaldi et al., 2000).

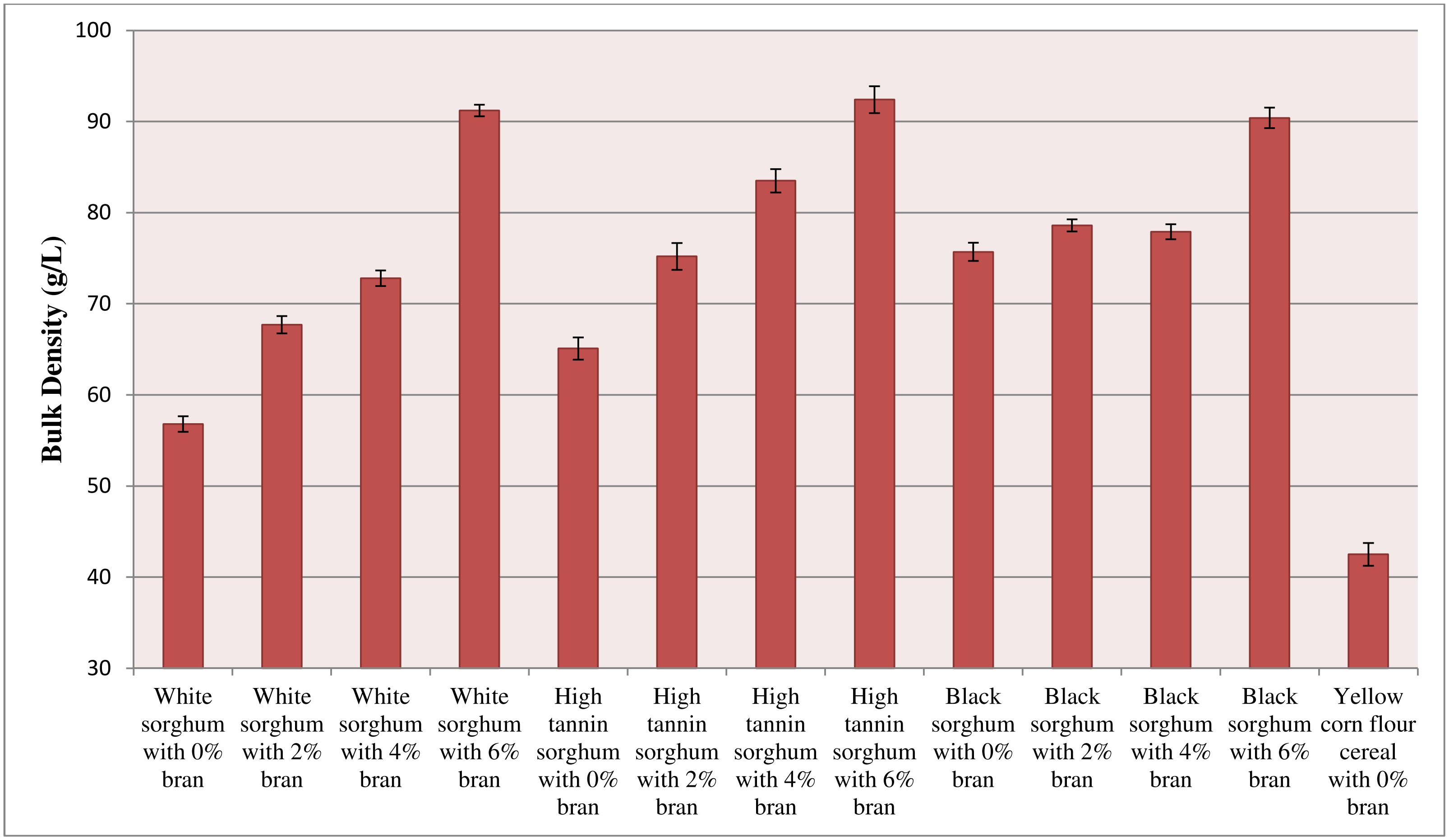


Figure 34: Bulk density of extrudates developed by using yellow corn flour, whole ground sorghum with and without high tannin sorghum bran (Experiment 4)

In this experiment, processing conditions were used to get whole ground sorghum extrudates with good expansion (Figure 35). Sorghum starch expands just like corn flour because of 50-75% starch (70-80% amylopectin and 20-30% amylase), which could allow gluten free whole grain breakfast cereals and snacks production. We could get more expansion with specialty sorghums with additional bran by changing the extrusion conditions but it would significantly reduce the antioxidant retention of extrudates. Desrumaux et al. (1998) mentioned that expansion of extrudates depends upon feed composition, extent of cooking and melt flow in the die. Fibers bind some of the moisture present in the matrix, thus reducing its availability for expansion (Onwulata et al., 2001). Gomez et al. (1988) extruded different varieties of decorticated sorghums at different moisture levels. They concluded that extrudates expansion index increased and bulk density decreased when sorghum was extruded with decreasing moisture content.

Keeping extrusion conditions and knife speed constant, the length of extrudates indicated how easily material flows through the die. Extrudate made from high tannin sorghum and 6% additional bran were longer than those made with reduced bran level. All white sorghum extrudates with additional bran had the same length, but for black sorghum, all treatments were elongated. Overall the expansion of whole ground black sorghum extrudates was quite different compared to other extrudates. This may be due to the sharp edges of black sorghum pericarp fractions which physically hinder air cell formation and expansion during the extrusion process by rupturing the air cell walls as mentioned by Austin (2008).

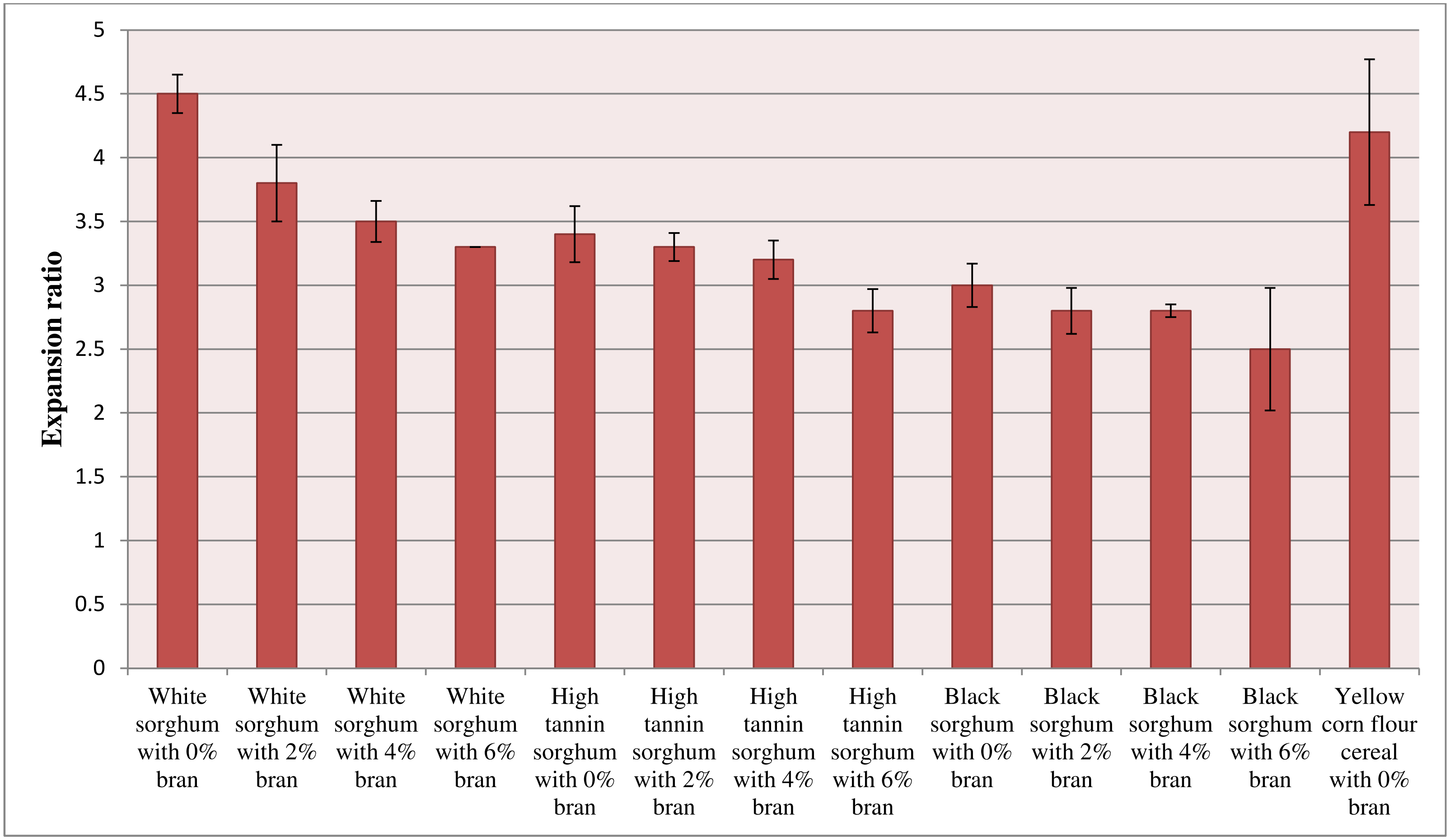


Figure 35: Expansion ratio of extrudates developed by using yellow corn flour, whole ground sorghum with and without high tannin sorghum bran (Experiment 4)

Extrudates made with whole ground high tannin sorghum and up to 4% high tannin sorghum were round in shape. Grenus et al. (1993) reported that radial and axial expansion of rice flour extrudates increased at 10% rice bran and decreased at higher levels (20 and 30%). Hsieh et al. (1988) added wheat and oat bran to corn meal up to 20% and 30% respectively. This increased the longitudinal expansion and bulk density, but decreased the radial expansion. At small concentrations inside the extruder, fiber molecules align themselves in the direction of flow to support the expanding matrix. On the other hand if the bran level increased above a critical concentration the fiber molecules disrupt the continuous structure of the melt and obstruct its elastic deformation during expansion. According to Acosta-Sanchez (2003) the length of extrudates was directly correlated to the specific mechanical energy (SME) received by the raw material. Melt viscosity determines the longitudinal expansion and thus higher SME resulted in a lower viscosity melt inside the extruder causing longer extrudates (Launay & Lisch, 1983).

4.7.4 Hardness (Maximum Compression Force) of Extrudates

Texture, one of the most important sensory attributes of extruded products is controlled by raw material formulation and extrusion condition. Maximum compression force of sorghum based extrudates varied significantly ($P<0.05$) between 14 and 18.3N. Hardness of extrudates decreased with increasing bran level except black sorghum extrudates.

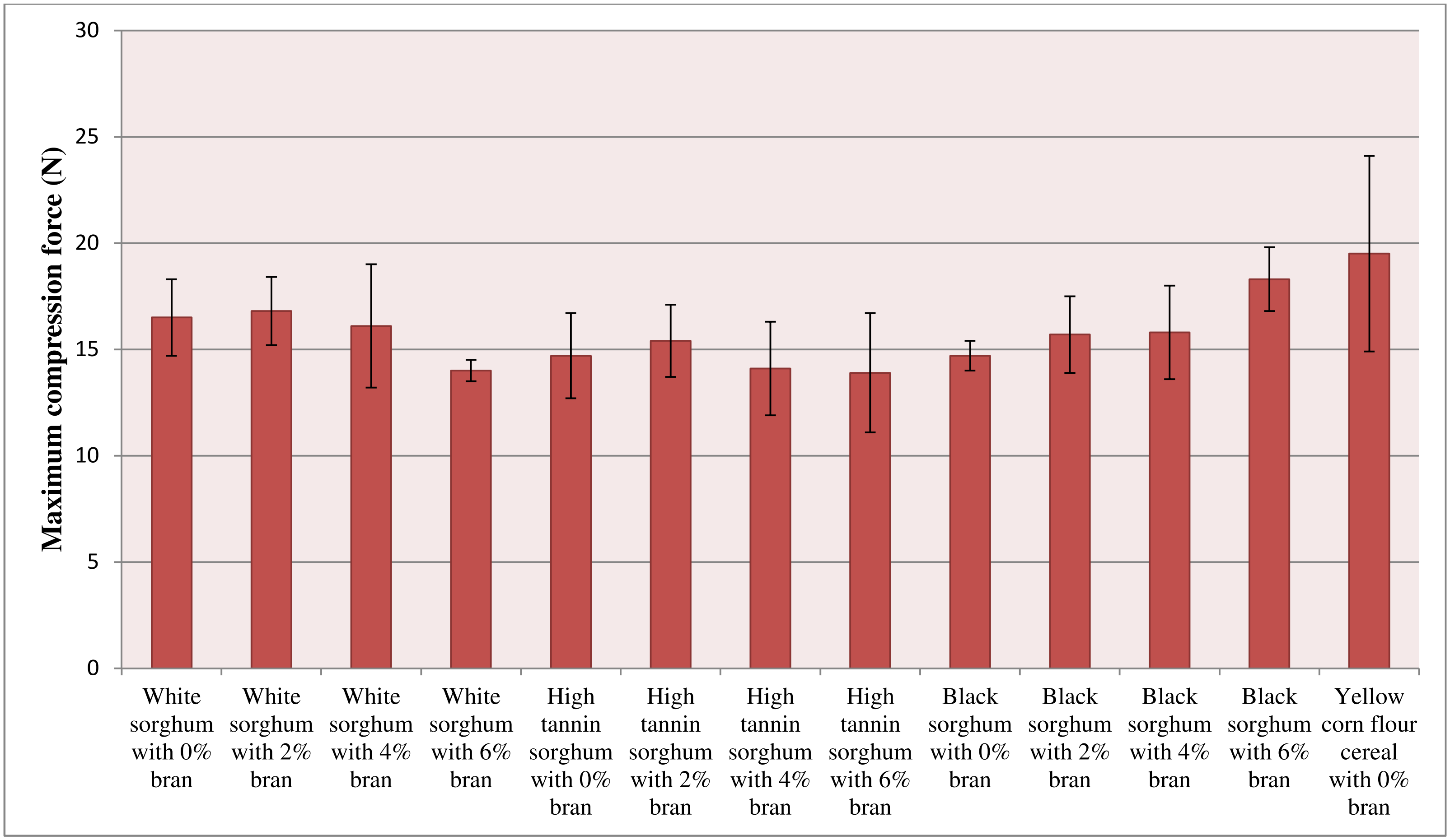


Figure 36: Hardness of extrudates developed by using yellow corn flour, whole ground sorghum with and without high tannin sorghum bran (Experiment 4)

Hardness of extrudates is also related with the extent of gelation of starch present in the extrudates. In this experiment the water flow to the extruder barrel was 4.1 kg /hr for all treatments. Extrudates without any bran in formulation used this water and produced an extrudate with compact texture without any holes or cracks. While for an extrudate with high bran percentage this water was not sufficient to hydrate starch and bran completely thus extrudate produced was not as compact as without bran. Example of this is yellow corn flour extrudate, which required highest (19.5N) maximum compression force to crush (Figure 36, Table 22). Previous studies have also reported that the hardness of extrudate increases as the feed moisture content increases (Badrie & Mellowes, 1991) and we already knew that fibers bind some of the moisture present in the matrix (Onwulata et al., 2001). Therefore the feed moisture in combination with fiber content of formulation played an important role in the hardness of extrudates.

The maximum compression force was decreased for extrudates made from white and high tannin sorghum with additional bran levels. In a previous experiment (2) we had similar results, where maximum compression force was decreased from 26.6 to 23.7N when an additional 10% whole ground white sorghum and 5% high tannin bran was added in formulation. There was more force required for crushing black sorghum extrudates with additional 6% bran (Figure 36). This was probably because of dense cell walls of these extrudates as Austin (2008) found that black sorghum pericarp (bran) is hard as compared to white and high tannin sorghums. Hardness of extrudates is also associated with the particles size of feed. High tannin sorghum bran was difficult to grind in to fine particles, therefore by increasing the bran percentage in whole ground sorghum flour the proportion of larger particles was increased. By increasing particle size of feed, extrudates with slightly larger cell sizes, lower cell density and soft texture were produced (Altan et al., 2009). The hardness of extrudate is a perception of consumers and correlated with the expansion ratio and cell structure of extrudates (Ding et al., 2005).

4.7.5 Bowl Life of Extrudates

Bowl-life is a sensory parameter related to the length of time a breakfast cereal retains its crispness after being immersed in milk (Puppala, 1998). Loss of breakfast cereal crispness in the bowl has been described as a rapid non-equilibrium dual mass transfer process, which involves the concurrent uptake of moisture and the loss of soluble solids (Machado et al., 1999). In these experiments difference in bowl life was observed with the level of bran used. Bowl life of extrudates varied significantly between 5.3 to 14 min (Table 22). Bowl life of extrudates was low for the extrudates without additional bran and increased as additional bran was added. White sorghum extrudates without additional bran had the shortest (5.3±0.6 min) bowl life. It increased to 8.7±0.6 min for the white sorghum extrudates with 6% additional bran.

Bowl life of high tannin sorghum extrudates without additional bran was 10.3±0.6 min, which increased to 14 min with 4% additional bran and then decreased to 10.3 min with 6% high tannin bran. Bowl life of black sorghum extrudates with 0, 2, 4 and 6% bran was 11, 10.7, 12 and 10.7 min respectively (Figure 37). Bowl life of yellow corn flour based extrudates (control) was 5.7±0.6 min.

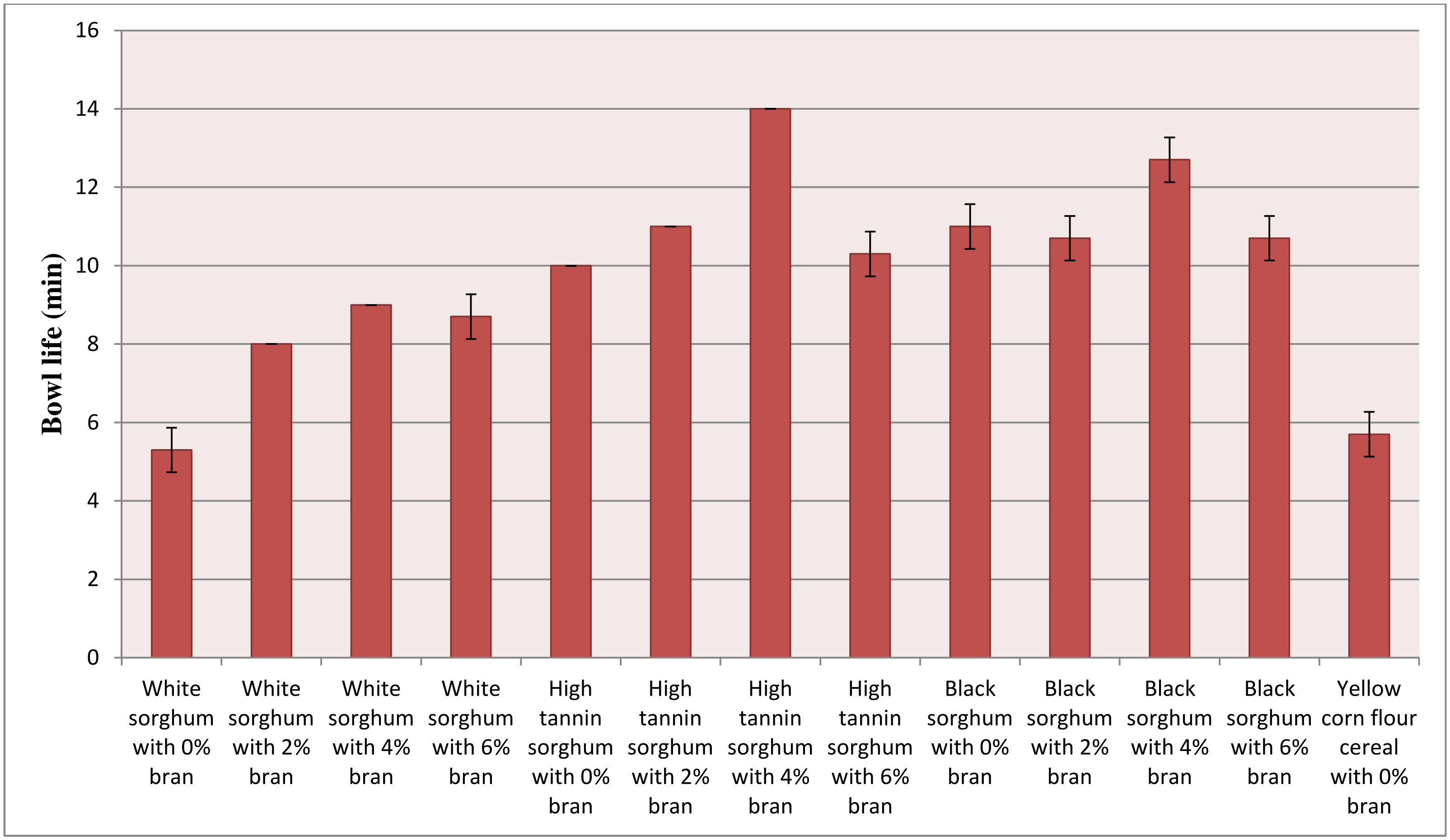


Figure 37: Bowl life (min) of extrudates developed by using yellow corn flour, whole ground sorghum with and without high tannin sorghum bran (Experiment 4)

Increasing bran contents in sorghum based breakfast cereals and snacks, up to a certain limit, caused premature rupture of gas cells of melt as it emerges from the die. These cracks or fine holes in the outer walls of extrudates facilitates the absorption of milk and reduces bowl life. Dietary fiber incorporation into extruded puffed snack foods and breakfast cereals limited puffing, reduced crispness and decreased bowl life (Dhananjay et al., 2006). A positive correlation between water soluble index and expansion ratio (R^2=0.89) indicated that more expansion ratio provided a larger surface area for water to interact with starch and other soluble components (Lee et al., 1999). In other words, more expanded cereals had less bowl life.

There are many ways to increase bowl-life through changes in the manufacturing process, cereal morphology, ingredient formulation or the addition of surface coatings. For example, increasing the glass transition temperature of high-sugar cereals by formulating with higher molecular weight polysaccharides extends bowl-life (Nelson & Labuza, 1993). Breakfast cereals were prepared with whole-ground white sorghum and 5–10% high-tannin sorghum, the bowl life of cereals was increased up to 18 min (Asif et al., 2010). Yanniotis et al. (2007) found that fiber increased the hardness of extrudates and decreased the porosity which increased the bowl life of extrudates.

4.7.6 Water Soluble and Absorption Index of Extrudates

Water soluble and water absorption index was calculated for selected extrudates. WSI is used as a measure of starch degradation. Lower WSI means minor degradation of starch occurred which leads to less soluble molecules in the extrudates (Hernandez-Diaz et al., 2007). Higher moisture during extrusion decreased protein denaturation and starch degradation. There was a significant ($P<0.05$) difference in the WSI of extrudates (Table 22). White sorghum extrudates with 0% bran had 52% WSI, which decreased to 20.6% when 6% bran was in the formulation. Similarly in high tannin sorghum extrudates without and with 6% bran WSI were 31.9, and 20.8% respectively. Lower WSI of black sorghum extrudates without additional bran is (25.8%) due to its floury endosperm, which required more water to hydrate but we kept feed water constant for all treatments. The WSI was significantly higher for the extrudates without additional bran and decreased as additional bran was added. A positive correlation (R^2=0.8982) between WSI and expansion ratio was found (Figure 38). This indicated that the more expansion ratio provided a large surface area for water to interact with starch and other soluble components (Lee et al., 1999). In other words, more expanded cereals have less bowl life.

WAI indicates the ability of extrudates to absorb water. It depends upon the availability of hydrophilic groups which bind water molecules and on the gel formation capability of macromolecules. There was a significant ($P<0.05$) difference in the WAI of white sorghum extrudates without and with 6% additional bran. There was non-significant difference in WAI of high tannin and black sorghum extrudates with and without additional bran. WAI of extrudates ranged from 2.5 to 5.9 g/g as particle size of feed was increased, WAI value decreased. Our results suggest that during extrusion starch and bran undergo high shear fragmentation which leads to increased dextrinization and improved water absorption capacity. Because the water flow to the barrel was constant and it too low for high tannin and black sorghum extrudates to completely gelatinize starch-bran composite but enough for white sorghum extrudates to gelatinize. Badri & Mellowes (1991, 1992) reported similar findings where WSI of several extrudates decreased and WAI increased with increasing moisture level and wheat bran. The WAI estimates the amount of water absorbed by starch and can be used as an index of gelatinization because disrupted starch granule bind more water (Ding et al., 2006). Hashimoto & Grossmann (2003) reported that by increasing fiber level and decreasing starch contents, the WAI of extrudates increased. However an increase in bran contents tended to decrease the WSI.

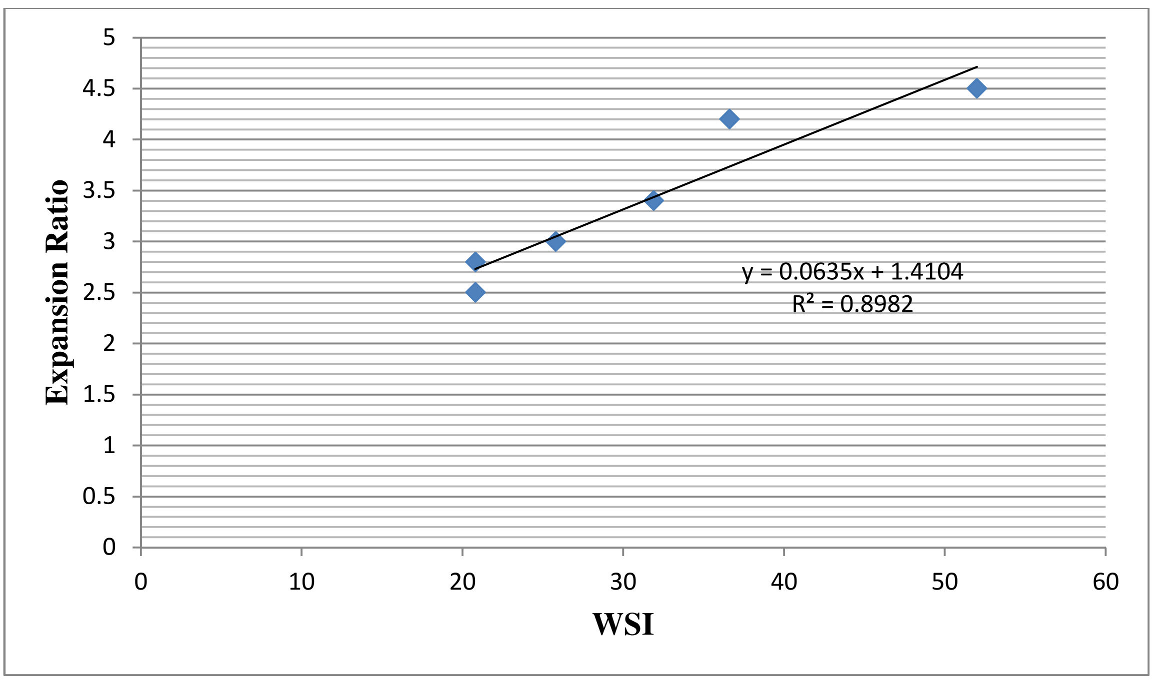


Figure 38: Correlation between expansion ratio and water soluble index of extrudates

4.7.7 Effect of Extrusion on Phenolic Compounds of Extrudates

All sorghums contain phenolic compounds but the amount present in any particular cultivar is influenced by its genotype and the environment in which it is grown (Hahn et al., 1984). High tannin sorghums have consistently been ranked as high in antioxidant activity with levels comparable to blue berries. These sorghums produce condensed tannins in the outer layer of the sorghum kernel. Therefore, the whole grain must be used to obtain health benefits. In addition, the tannins and related compounds are easily concentrated in sorghum bran which can be obtained by decortication of the grain. The bran is high in dietary fiber, condensed tannins and natural dark brown color. Thus, it is possible to utilize whole grain and/or bran of the tannin sorghums to produce food products with improved levels of important compounds that are associated with health benefits. Consumption of tannin sorghums may be associated with reduced risk of certain types of cancer especially colon cancer.

The phenols in sorghums fall under two major categories; phenolic acids and flavonoids. The phenolic acids are benzoic or cinnamic acid derivatives (Waniska & Rooney, 2002), whereas the flavonoids include tannins and anthocyanins as the most important constituents isolated from sorghum (Krueger et al., 2003). High tannin sorghum bran had significantly ($P<0.05$) higher [33 mg gallic acid equivalents (GAE)/g] total phenols, followed by high tannin and whole ground black sorghum flours (Table 23). There was a non-significant difference of total phenols in high tannin and whole ground black sorghum (Figure 39). The sorghum types with pigmented testa layers had higher levels of total phenols. Sorghum with a thick pericarp had higher total phenols than those with a thin pericarp (Beta et al., 1999).

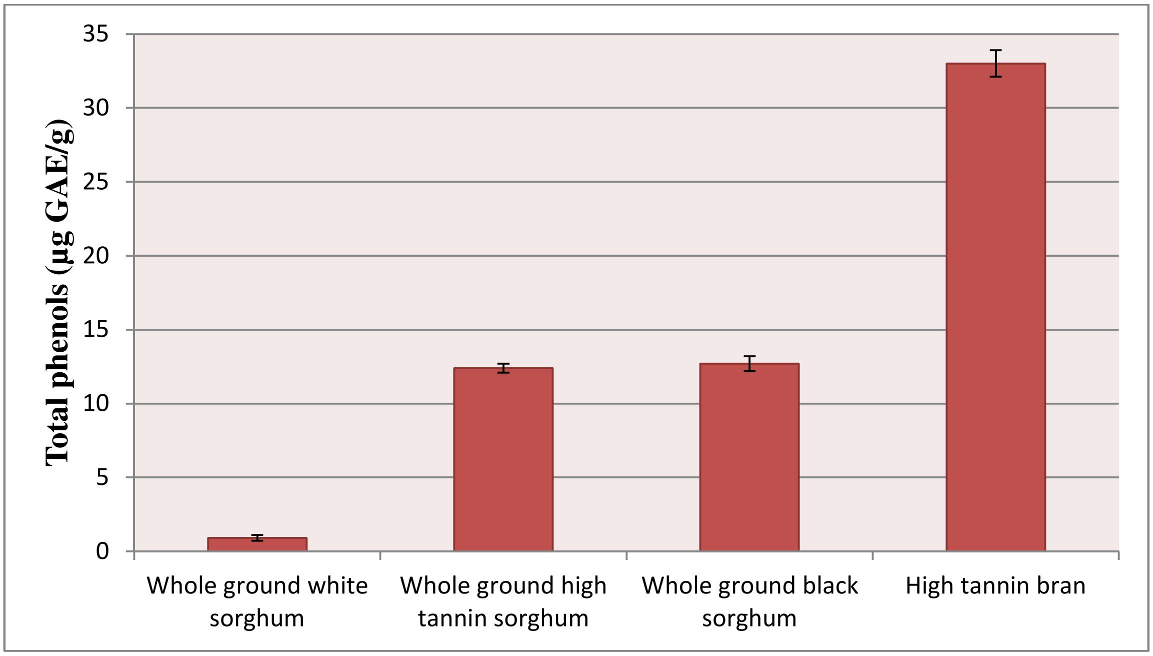


Figure 39: Total phenols (µgGAE/g) of whole ground white, high tannin and black sorghum grain and high tannin bran

The total phenol levels differed significantly (P<0.05) between the different sorghum types, with high tannin sorghums the highest, followed by black and white. Total phenol in composite flours of different sorghums increased significantly (P<0.05) by increasing the bran substitution. This increase was not significant for black sorghum composite flour (Figure 40).

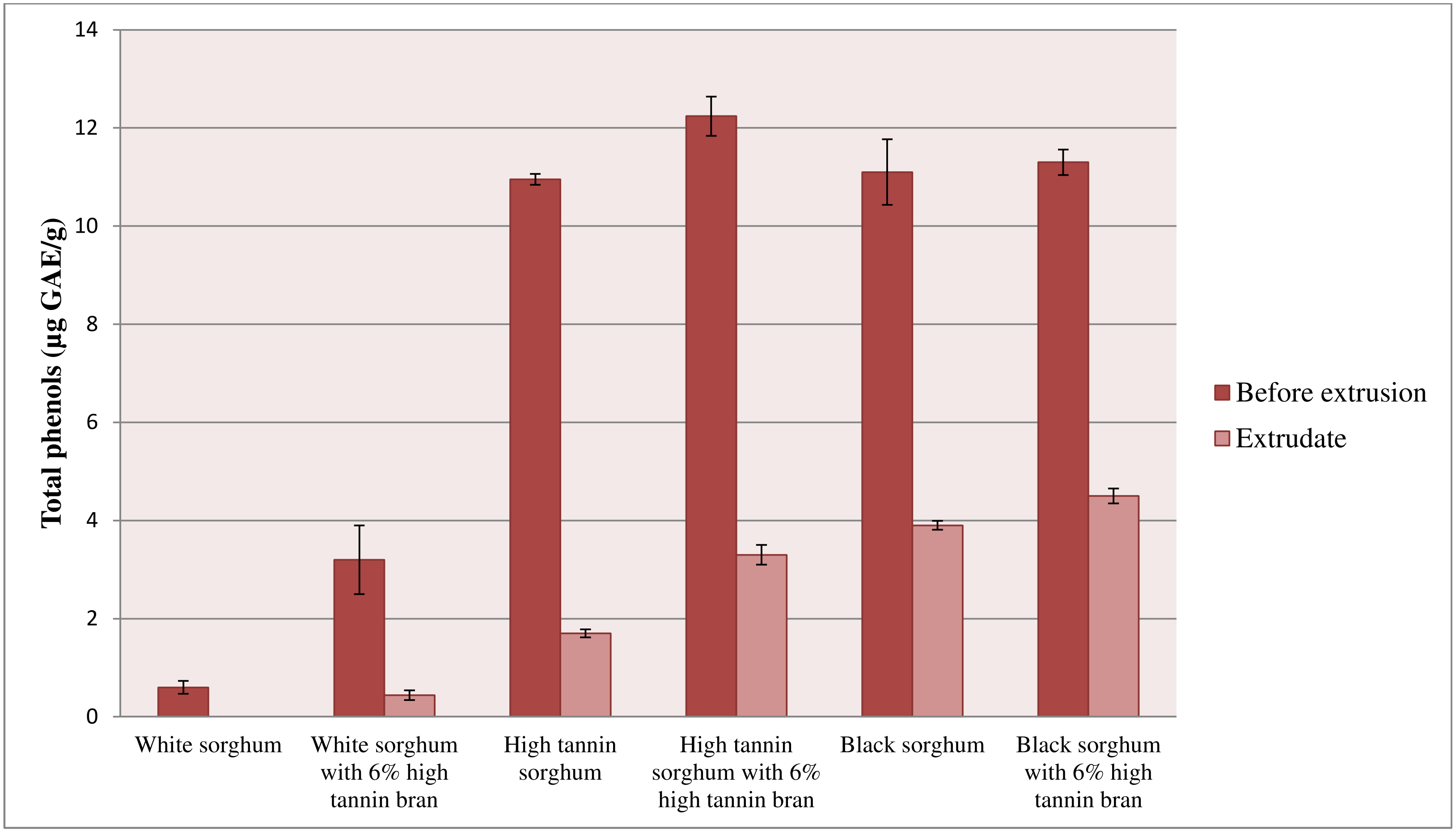


Figure 40: Total phenols of sorghum composite flours with and without bran before and after extrusion

Highest total phenols (12.2±0.4) were estimated in high tannin sorghum flour with 6% additional high tannin bran, while black sorghum flour with and without additional bran had 11.3 and 11.1 mg GAE/g of total phenol respectively. White sorghum flour without and with 6% additional bran had 0.6±0.13 and 3.2±0.7 mg GAE/g total phenols respectively.

Extrusion cooking significantly reduced the measurable total phenols for all three types of sorghum. Extrudates with additional 6% high tannin sorghum bran had more total phenols than extrudates without it. Recovery of phenols in black sorghum extrudates with 6% additional high tannin sorghum bran was about 40%. High tannin sorghum extrudates with and without 6% additional bran had 16 and 27% total phenols recovery respectively. Polyphenols are decreased during processing, depending upon the feed stocks and process (Awika, 2003). Some researchers suggest that polyphenols may be more bioavailable than previously thought (Rechner et al., 2002; Ross and Kasum, 2002). Awika et al. (2003b) suggested that there could be fragmentation of procyanidins during extrusion cooking, which might increase bioavailability.

Table 23: Total phenols, antioxidant activity and tannin contents of sorghum products

	High tannin bran%	Total Phenols (µgGAE/g)	Antioxidant Activity (µmolTE/g)	Tannins (mgCE/g)
White sorghum mix before extrusion	0	0.6bc	2.6a	0.05a
	6	3.2e	38.7c	1.7b
White sorghum extrudates	0	0.0a	6.6ab	0.2a
	6	0.4bc	14.4b	0.2a
High tannin mix sorghum before extrusion	0	10.9g	169.8f	6.7de
	6	12.2h	183.9g	7.5ef
High tannin sorghum extrudates	0	1.7d	35.9c	0.8ab
	6	3.3e	56.0d	1.3b
Black sorghum mix before extrusion	0	11.1g	167.5f	5.7c
	6	11.3g	163.5f	6.4cd
Black sorghum extrudates	0	3.9f	42.9c	1.3b
	6	4.5f	67.0e	1.6b
White sorghum whole ground	-	0.9c	13.3b	0a
High tannin sorghum whole ground	-	12.4h	167.2f	7.8f
Black sorghum whole ground	-	12.6h	193.3g	8.9g
High tannin sorghum bran	-	33.0i	368.7h	23.2h

Values in each column with different letters are significantly different at P<0.05

Antioxidant activity of whole ground sorghums was significantly ($P<0.05$) different. The highest antioxidant activity (368.7±1.8 µmol TE/g) was in high tannin sorghum bran (30% decortication). In whole ground sorghums the lowest antioxidant activity was in white sorghum (13.3±2.2 µmol TE/g), while black sorghum was highest (193.3 ± 8 µmol TE/g). Antioxidant activity of high tannin sorghum flour was 167.2±11.8 (µmol TE/g) which was significantly lower than that of high tannin sorghum bran (Table 23, Figure 41).

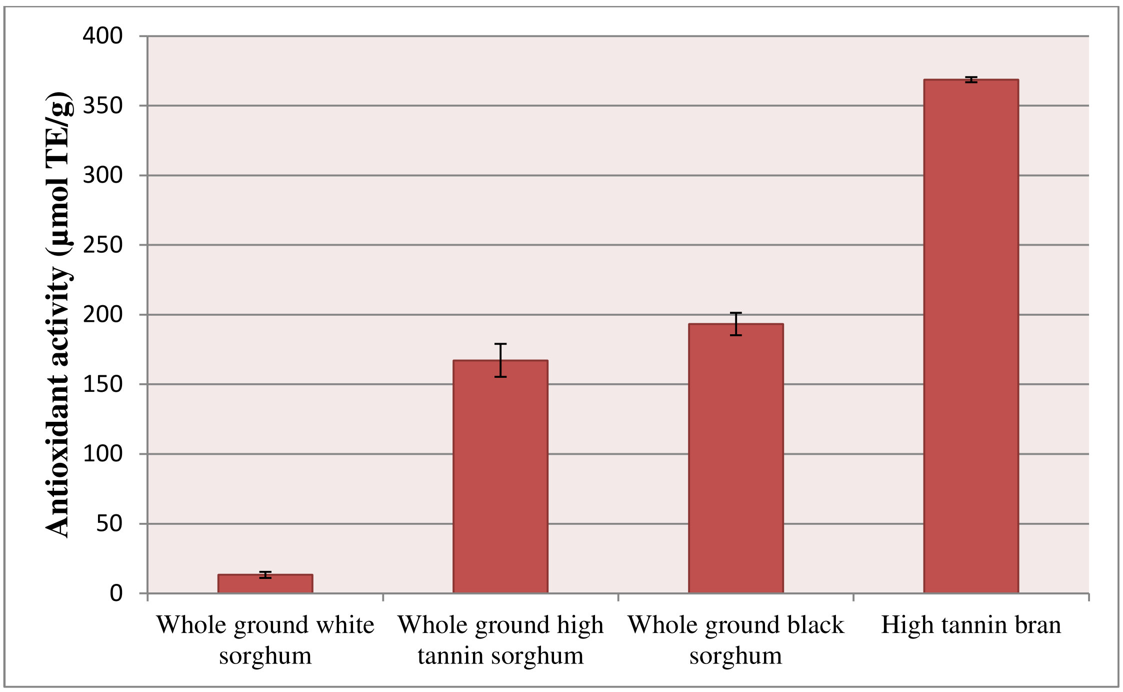


Figure 41: Antioxidant activity of different types of sorghum and high tannin sorghum bran

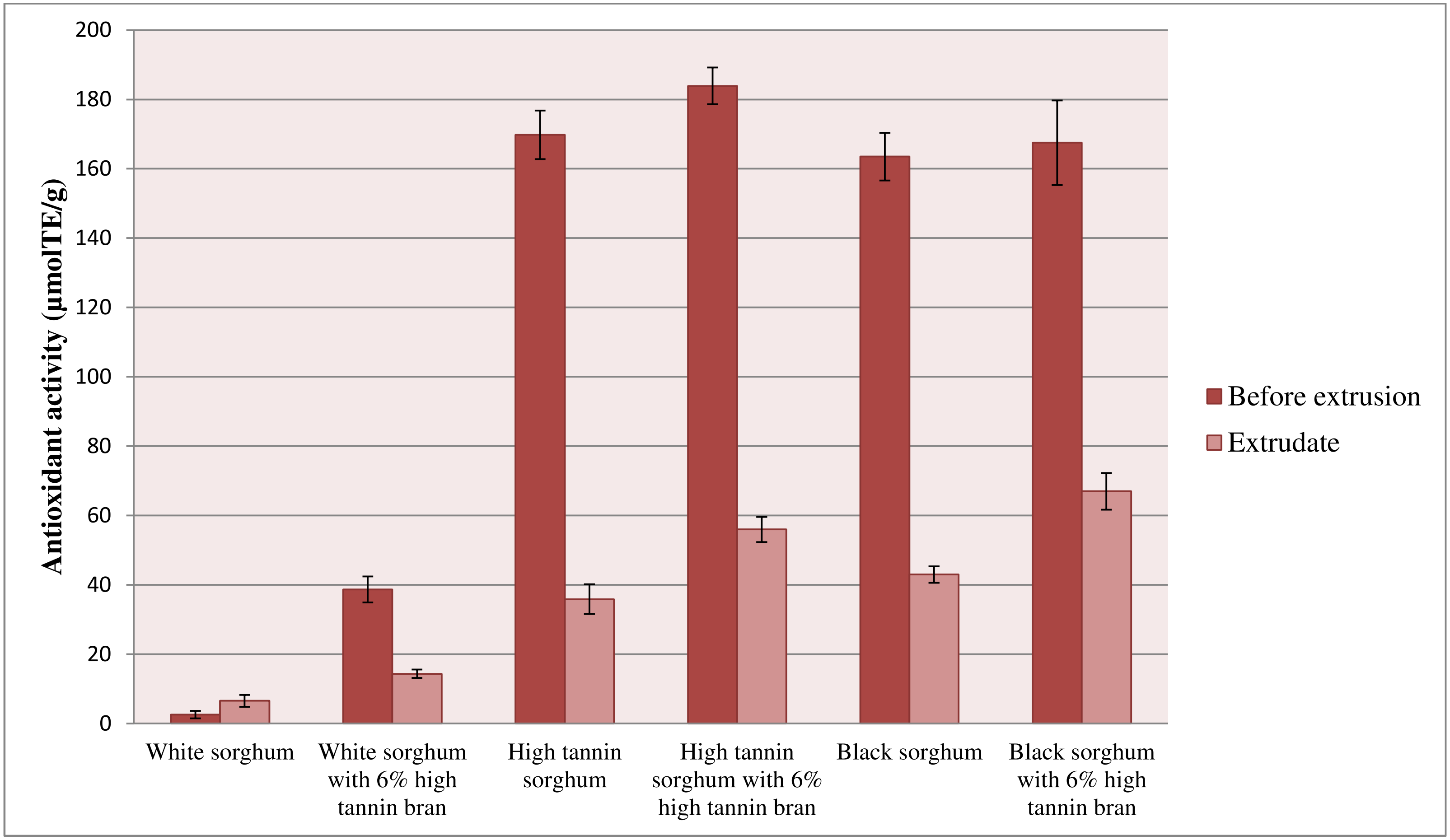


Figure 42: Antioxidant activity of sorghum with and without bran before and after extrusion

The antioxidant activity in tannin sorghum is contributed mainly by condensed tannins which have demonstrated higher free radical quenching ability *in vitro* than other phenolic compounds (Hagerman et al., 1998; Awika et al., 2003a). Most of these condensed tannins are concentrated in the pericarp therefore the bran fraction (30% decortication) is higher in antioxidant activity than whole grain flour.

All three types of sorghum (white, high tannin and black) composite flours with and without additional high tannin bran showed significant antioxidant activity before and after extrusion (Table 23, Figure 42). As the percentage of the antioxidant activity in whole ground composite flours, the extruded samples retained 21% and 26% for high tannin and black sorghum without additional high tannin bran respectively. The retention of the antioxidant activity percentage was significantly increased up to 30% in high tannin sorghum extrudates when additional 6% high tannin sorghum bran was added. Addition of high tannin sorghum bran in black sorghum composite flours also increased the antioxidant activity up to 40% in the extrudates. The significant reductions in antioxidant activity were higher for tannin sorghums compared to black and white sorghum types. The reduction in antioxidant activity in cooked high tannin sorghum can be attributed largely to the interaction of tannins with prolamins (Emmambux and Taylor, 2003).

The antioxidants retentions in these experiments were significantly lower compared to those of sorghum extrudates reported by Awika et al. (2003b), where 70-100% of antioxidant activity was retained. Awika et al. (2003b) used a single-screw friction type extruder compared to the co-rotating twin-screw extruder used in these experiments. The smaller fraction (particle) size used in this experiment probably increased the surface area of contact between composite flour components and extruder barrel which promoted interactions during extrusion lowering antioxidant activity. Extrusion involved high heat and shear conditions, which may have broken down the flavonoids and made them unavailable for antioxidant activity analysis. Ngwenya (2007) found that extrusion cooking significantly decreased antioxidant activity by 83 to 87% for sorghum products compared to the raw grains when twin screw extruder was used.

In white sorghum extrudates the antioxidant activity was increased compared to the composite flour. During extrusion the phenols are converted through cleavage and re-association to other compounds that are not detectable as phenols by the method used. These new compounds may retain most of antioxidant activity of original compounds. Awika (2003) found that all sorghum samples analyzed retained high antioxidant activity after different types of processing. The antioxidant activities in products depended largely on the antioxidant activates in the raw materials. Turner (2004) reported that extrudates containing 50% tannin bran retained the highest percentage of tannins and antioxidant activity at 49.2 and 82.6% respectively because they were least modified by the extrusion process. Phenols, tannin and antioxidant activity percentages in extrudates increased with bran levels, while antioxidant activity was increased up to 67% in extrudates containing 50% bran (Turner, 2004). Awika (2003) reported similar increases in antioxidant activity in breads and cookies fortified with increasing amounts of tannin and black sorghum bran. Antioxidant activity of sorghum was highly correlated with total phenols (R^2= 0.99) present (Figure 43).

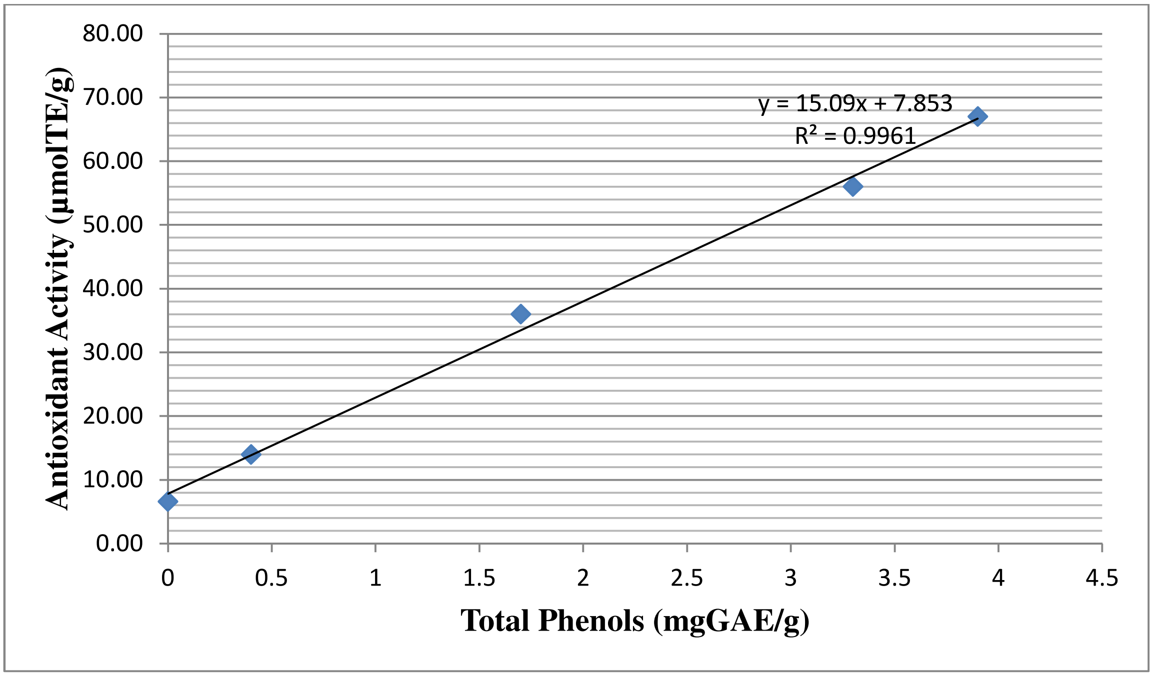


Figure 43: Correlation between antioxidant activity and total phenols in sorghum extrudates

Some sorghum varieties produce large amounts of tannins which makes them unique among the cereals (Serna-Saldivar & Rooney, 1995), however not all sorghum varieties contain condensed tannins. In this experiment, only high tannin and black sorghum had a pigmented testa with significant ($P<0.05$) condensed tannins (Figure 44, Table 23). White sorghum (without pigmented testa) did not show any detectable quantities of condensed tannins. While the high tannin sorghum bran contained 23.2 mgCE/g of condensed tannins.

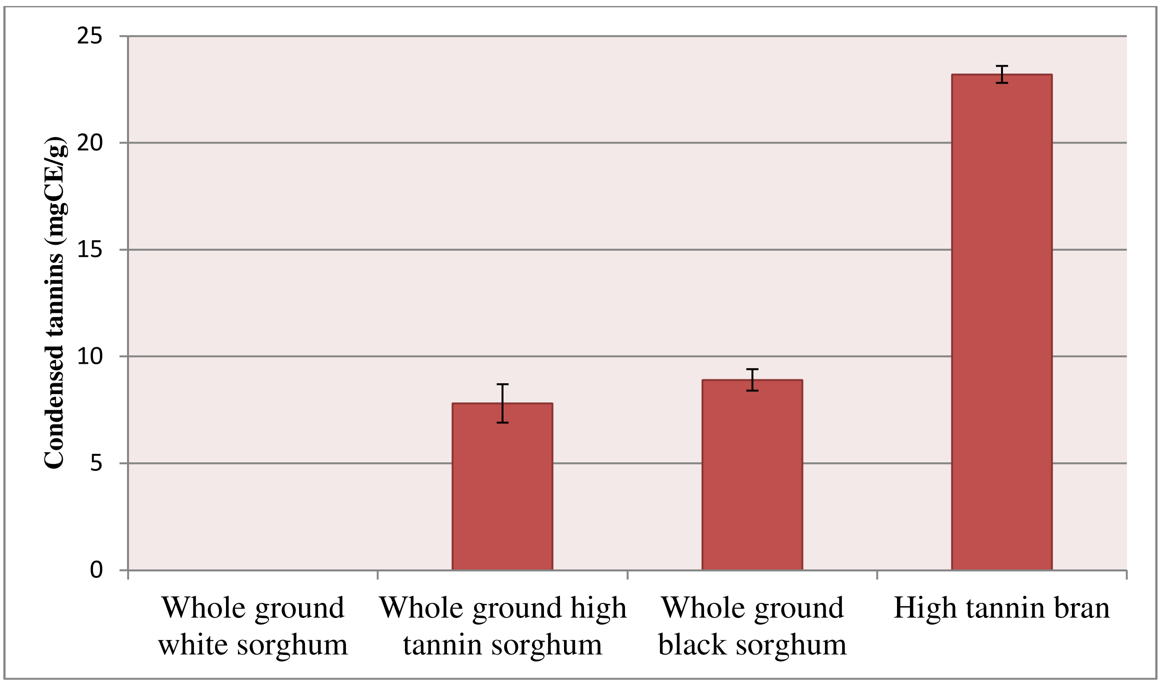


Figure 44: Condensed tannins of different types of sorghum and high tannin bran

Extrusion process itself and cultivar of sorghum, significantly reduced the total condensed tannins in the extrudates (Figure 45). The condensed tannins were reduced most (88%) in high tannin sorghum extrudates without additional high tannin sorghum bran. Black sorghum extrudates without any additional bran had 23% retention of condensed tannins, while extrudates with 6% additional high tannin bran had 25% retention of condensed tannins. Similarly Ngwenya (2007) found that extrusion cooking reduced measurable tannins by almost 97% in whole grain extrudates, while in decorticated extrudates tannins were not detected. The retention of condensed tannin in this experiment is low because high temperature and shear conditions involved were expected to breakdown the flavonoids. The smaller particle size of the composite flour, probably increased surface area of contact between composite flour and extruder components which promoted strong interactions during extrusion which ultimately caused lower retention of condensed tannins.

The third factor of reduced retention of condensed tannins could be the molecular interactions particularly with respect to interactions of condensed tannins with protein, which promote tannin-protein interaction. These interactions occur during the mixing and conveying inside a twin screw extruder. The twin-screw extruder has a lower shear level which may not create enough friction to depolymerize the condensed tannins into more extractable compounds. Single screw short barrel extruders create a high amount of friction and shear which may cause de-polymerization of the condensed tannins and their conversion to low molecular weight oligomers that were more extractable (Awika et al., 2003b).

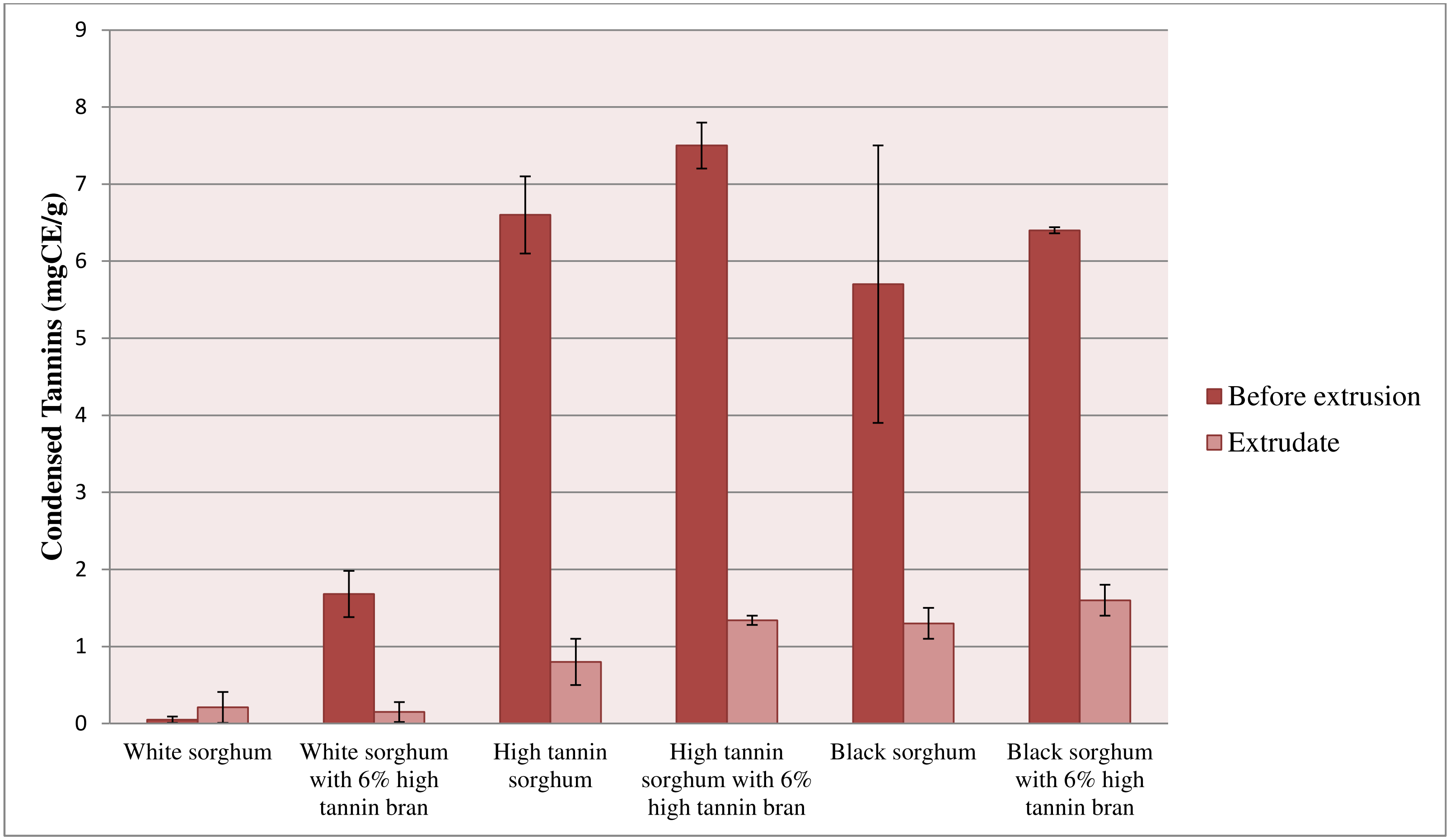


Figure 45: Condensed Tannins of sorghum before and after extrusion

Dlamini et al. (2009) stated that extruded sorghum porridges have decreased condensed tannin oligomers and polymers compared to traditional cooked porridges and grain. Hence, the antioxidant activity of phenolics in extruded sorghum porridges may be more readily available than in the conventionally cooked porridges (Dlamini et al., 2009). Hagerman et al. (1998) demonstrated that tannins, even when bound to protein, were still capable of acting as radical scavengers; this property was regarded as being of particular importance in the gastrointestinal tract where the tannin-protein complexes could act as free radical sinks. Condensed tannins present in the sorghum extrudates had a direct correlation (R^2=0.946) with the antioxidant activity of them (Figure 46).

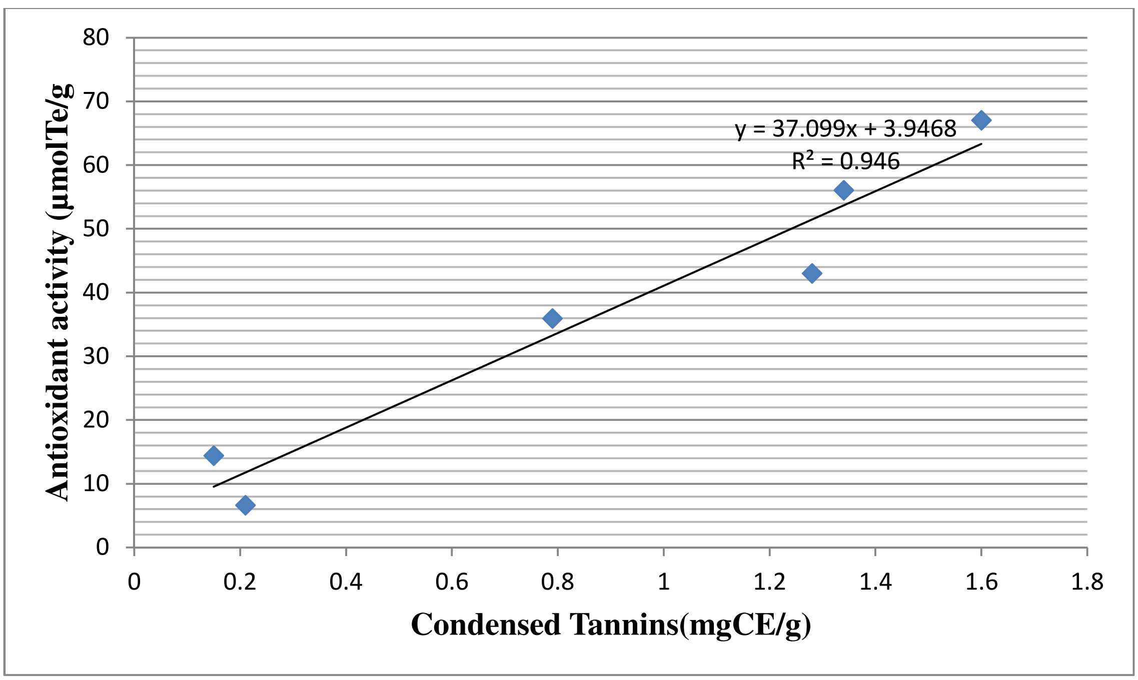


Figure 46: Correlation between antioxidant activity and condensed tannins in sorghum extrudates

4.7.8 Effect of Phenolic Compounds on *In Vitro* Starch Digestibility

Starch digestibility is affected by sorghum type, the extent of starch protein interaction, presence of tannins, the physical form of the granule, and the type of starch (Rooney & Pflugfelder, 1986). Phenolic compounds complex with proteins (Riedl & Hagerman, 2001) and carbohydrates (Naczk et al., 2006), generating insoluble polymers. Tannins bind sorghum kafirins (prolamins rich proteins) reducing protein availability, and consequently lower and slower starch digestibility of tannin sorghum.

In this experiment, the effect of extrudates of sorghum was determined on starch digestibility and compared with corn flour extrudates. Extrudates of white, high tannin and black whole ground sorghums with and without additional high tannin bran were used. In all types of sorghum extrudates, highest total starch (73.38%) was in high tannin sorghum extrudates. The whole black sorghum had significantly lower total starch (60.18%) compared to other sorghum types. Corn flour extrudates had 78% total starch which was significantly higher than total starch of sorghum extrudates (Table 24).

All sorghum extrudates had slower rates of starch digestion compared to corn flour extrudates. Less starch was hydrolyzed in sorghum extrudates compared to corn flour extrudates in 16 hrs. In corn flour extrudates the starch hydrolyzed in 0.5 hr was significantly higher (68.2g) than the starch hydrolyzed in sorghum extrudates. In white sorghum extrudates, starch hydrolyzed in 0.5 hr was 16.7% of starch present. This was not significantly different from starch hydrolyzed (16.2%) in white sorghum extrudates with additional 6% high tannin bran (Figure 47).

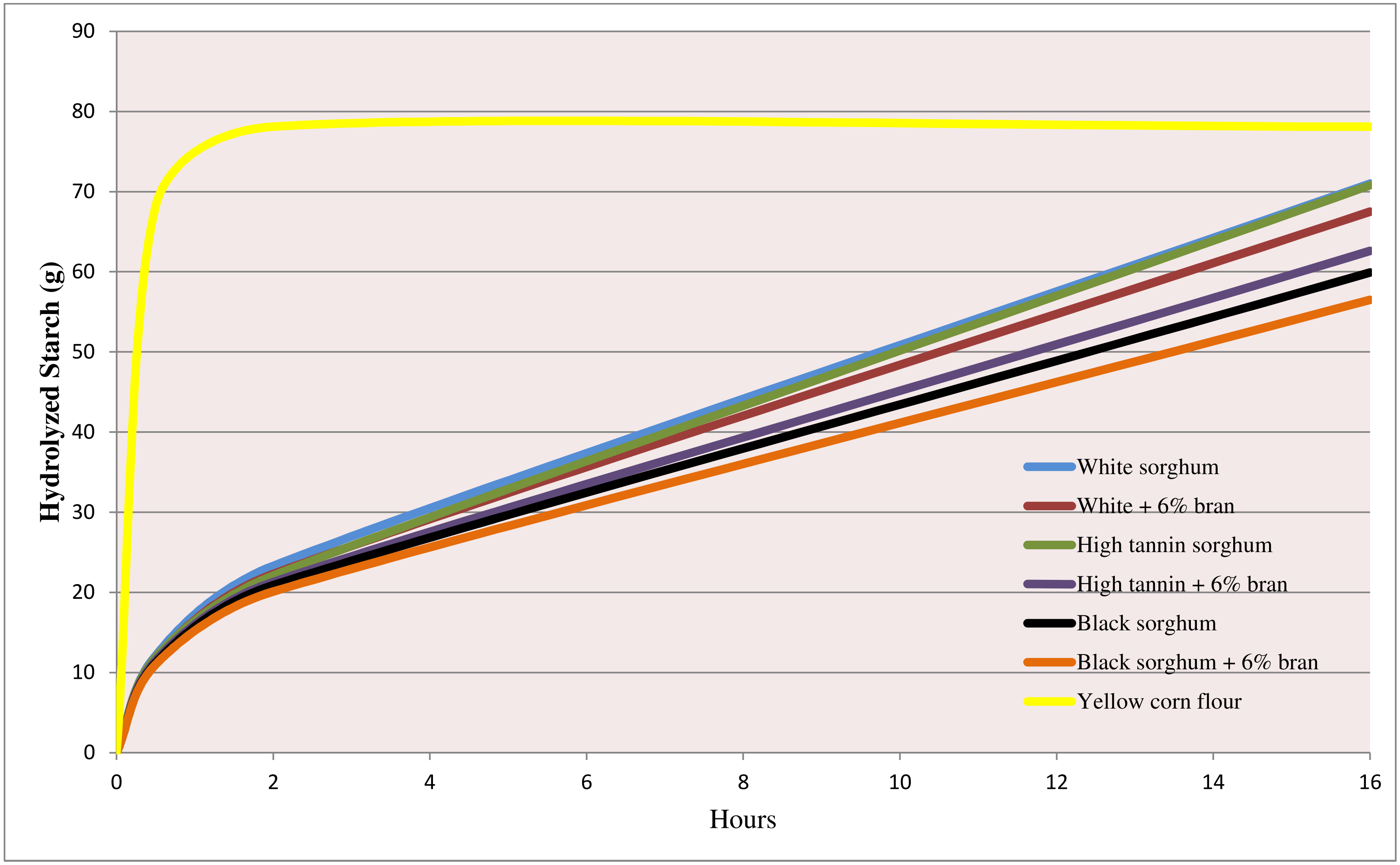


Figure 47: Digested starch (g db) of extrudates of yellow corn flour and sorghums, with and without tannin bran at different time intervals (Experiment 4)

Similarly starch hydrolyzed in high tannin sorghum extrudates with and without additional 6% bran was 16.2 and 17.4% respectively which was not significantly different. In black sorghum extrudates with and without addition of bran starch hydrolyzed in 0.5hr was 17.9 and 18.6% respectively. Therefore, starch percentage hydrolyzed in 0.5hr in all types of sorghum extrudates was similar to each other but overall it was significantly lower than that of corn extrudates.

There was no significant difference in starch digestibility of three types of sorghum with and without bran after 2 hrs (Figure 48). They were significantly different from the starch hydrolyzed (99.6%) in corn flour extrudates. In white sorghum extrudates 32.2% of starch was hydrolyzed in 2 hrs while in white sorghum extrudates with 6% bran, 30.9% of starch was hydrolyzed. In high tannin sorghum extrudates with and without additional bran 32.3 and 30.1% of starch was hydrolyzed respectively.

After 16 hrs, there was a significant difference in the starch digestibility of all types of extrudates. About 78.1g (99.6%) of total starch in corn flour extrudates was hydrolyzed which is significantly higher than that of high tannin sorghum extrudates, in which 70.8g (96%) of total starch was hydrolyzed. For black sorghum extrudates with and without 6% additional extrudates was 92 and 99.5% starch hydrolyzed respectively. On the other hand the percentage of starch hydrolyzed in white sorghum extrudates with 6% high tannin bran was lower (93.5%) than that of white sorghum extrudates without additional bran (98.2%).The sorghum starches were hydrolyzed at a slower rate than corn starch. Whole ground high tannin sorghum extrudates had the slowest starch hydrolysis. Addition of high tannin sorghum bran significantly reduced starch digestion.

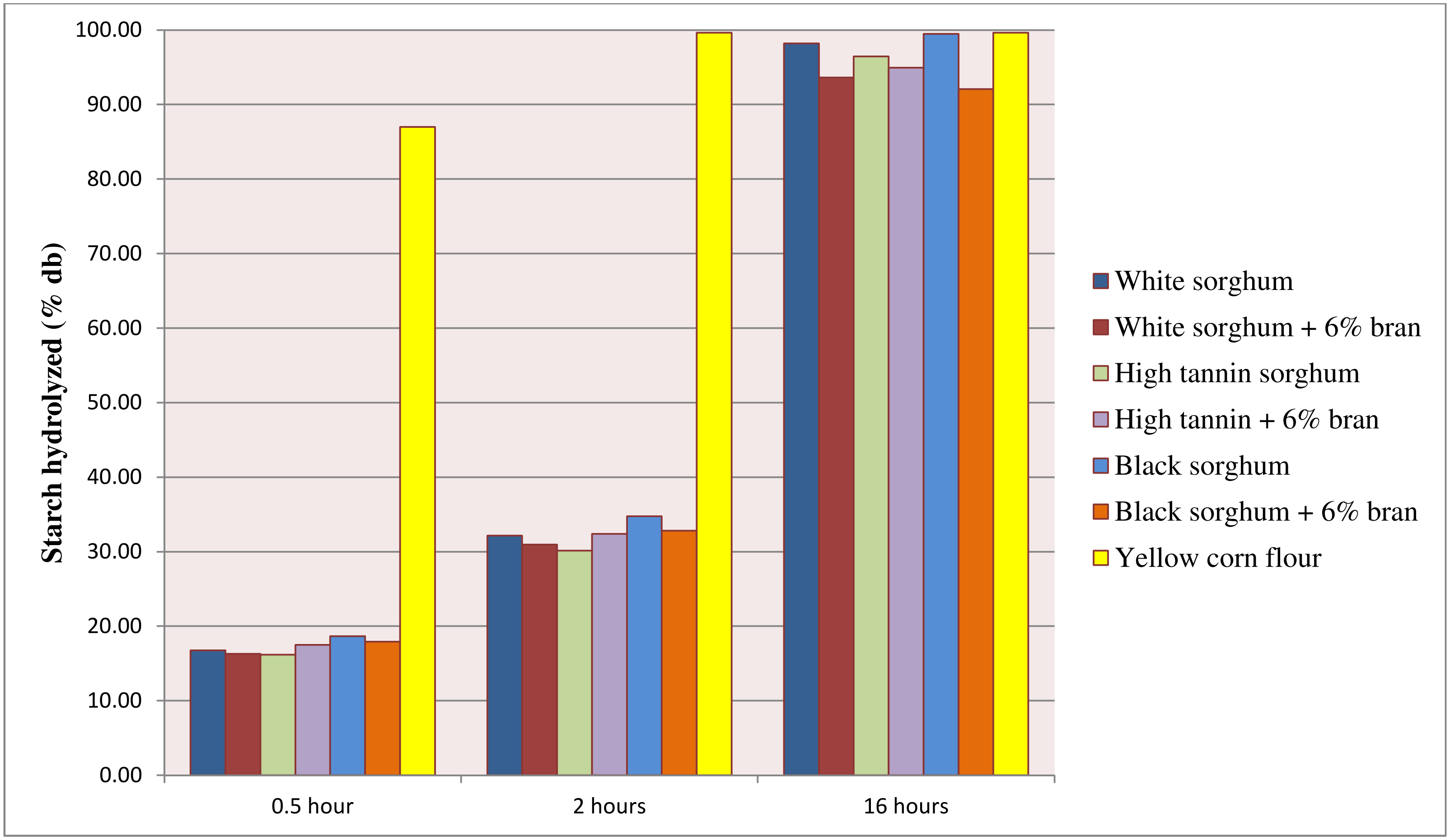


Figure 48: Starch *(in vitro)* digestibility (%bd) of sorghum and corn extrudates at different intervals (Experiment 4)

Table 24: *In vitro* starch digestibility of extrudates at different time intervals

Sorghum Extrudates	Total starch*	Starch Hydrolyzed (*) after			Undigested
		0.5 hr.	2 hrs.	16 hrs.	Starch*[1]
White	72.3c	12.1 ± 0.6a	23.3 ± 2.3a	71 ± 0.7c	1.3
White + 6% bran	72.1c	11.7 ± 0.4a	22.3 ± 2.2a	67.5 ± 1.1c	4.6
High tannin	73.4c	11.9 ± 0.8a	22.1 ± 2.3a	70.8 ± 5.6c	2.8
High tannin + 6% bran	65.9b	11.5 ± 0.5a	21.3 ± 2.2a	62.6 ± 2.1b	3.3
Black	60.2a	11.2 ± 0.4a	20.9 ± 1.4a	59.9 ± 0.7ab	0.3
Black + 6% bran	61.4a	11 ± 0.5a	20.1 ± 2a	56.5 ± 1.2a	4.9
Yellow corn flour	78.4d	68.2 ± 1.2b	78.1 ± 0.9b	78.1 ± 0.8d	0.3

*g/100g db
[1] Difference between total starch and starch hydrolyzed in 16 hr.
Values in each column with different letters are significantly different at $P<0.05$

Condensed tannins reduce the starch digestibility, because extrusion facilitates association of condensed tannins with starch and protein reducing the digestion of both (Duodu et al., 2003). In whole ground black sorghum, the bran's sharper particles may have physically disrupted the continuous matrix that holds starch-protein matrix together (Austin, 2008) in extrudates, creating weak points, making the extrudates more susceptible to α-amylase, which gave significantly ($P<0.05$) higher starch digestibility after 16 hrs. Addition of high tannin bran in white, high-tannin and black whole ground sorghum significantly ($P<0.05$) reduced starch digestibility.

Interaction between condensed tannins and sorghum proteins reduces both protein and starch digestibility (Daiber, 1975; Davis & Hoseney, 1979; Butler et al., 1984; Serna-Saldivar & Rooney, 1995; Emmambux et al., 2004). In this experiment, enzyme was used to analyze both total starch and starch digestibility and it is probable that tannins complexed with kafirins and starch in soft and hard endosperm during extrusion, which resulted in lower starch digestibility. These results agree with the findings of Austin (2008) when she studied the effects of sorghum phenols on starch digestibility of hard and soft endosperm porridges. She found that tannin sorghum were most effective for lowering starch digestibility, EGI and increasing RS contents of porridges, when used as whole grains, brans, and bran extracts. However black bran structure and its larger particle size distribution prevented anthocyanins from reducing EGI of porridges when they were added to hard and soft endosperm flours in the form of brans.

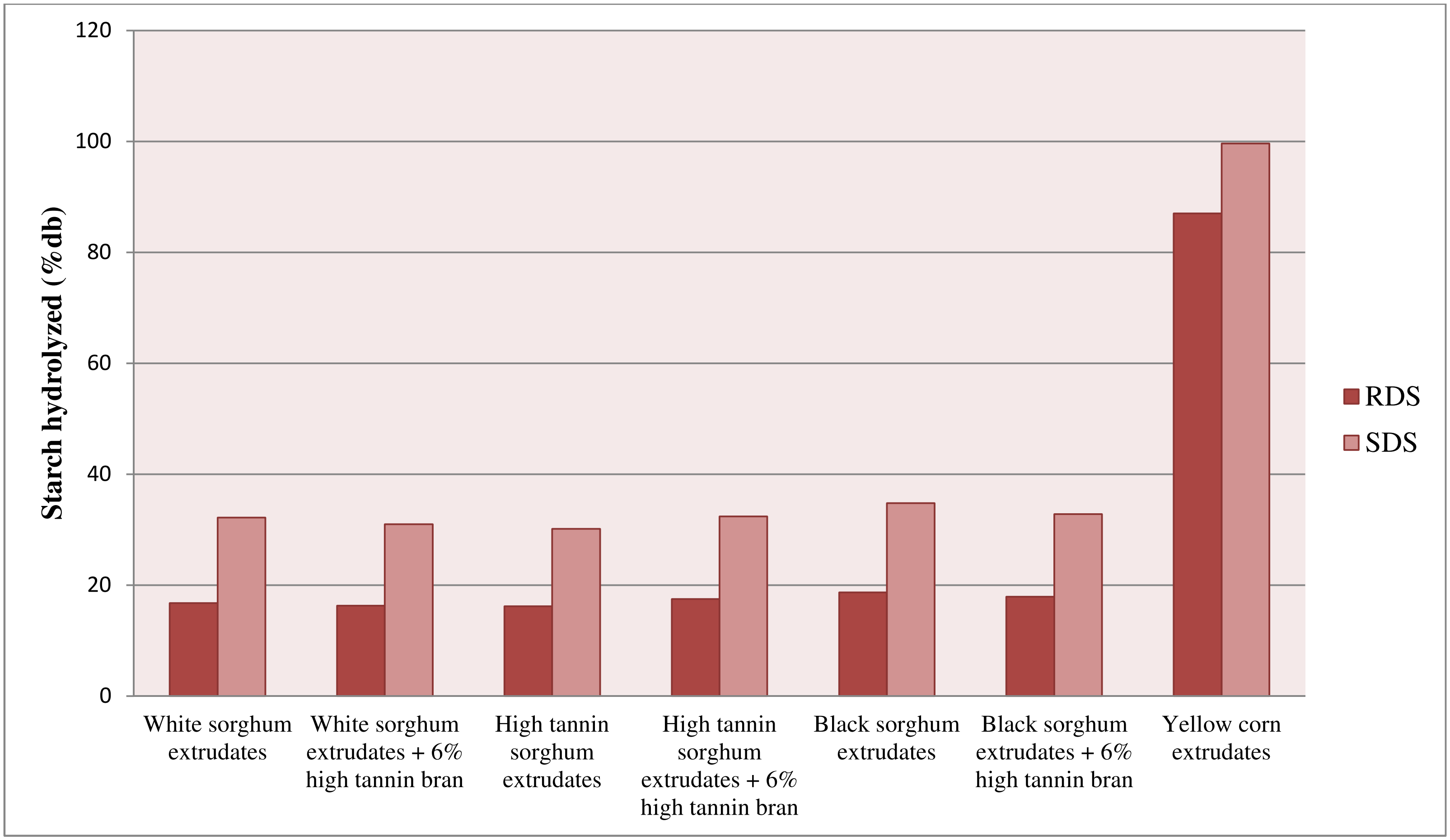


Figure 49: Rapidly digestible starch (RDS), and slowly digestible starch (SDS) of extrudates

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated from the *in vitro* starch digestion at 0.5 and 2hr of enzymatic incubation respectively (Figure 49). The RDS was high for the corn flour extrudates and was significantly lower for all sorghum extrudates. The SDS for sorghum extrudates with and without additional 6% bran was not statistically different from each other. But it was significantly higher for corn flour extrudates. The RDS of all the sorghum extrudates were statistically similar; however the SDS fraction was significantly different. RDS has been used as an alternative method to evaluate starch digestion. When the RDS fraction is high, the starch is considered rapidly digested, giving a high glycemic response *in vivo* (Rosin et al., 2002).

There was a negative correlation between the RDS and tannin contents (R^2=0.62). Increasing the tannin contents in the extrudates decreased the digestibility of starch (Figure 50). But not all types of sorghum contain tannins therefore incomplete starch gelatinization could also slow starch digestibility. A positive correlation between RDS and expansion ration (R^2=0.77) indicated that the most expanded extrudates were more rapidly digested by α–amylase (Figure 51).

Duodu et al. (2003) reviewed the factors affecting sorghum protein digestibility, which were broadly categorized as exogenous and endogenous factors. The former involves interactions of proteins with non-protein components like starches. The starches and sugars in sorghum are released more slowly than in other cereals (Klopfenstein et al., 1995) which could be beneficial to diabetics. Mesa-Stonestreet et al. (2010) mentioned that corneous part of endosperm of sorghum contains abundant protein bodies that surround the starch granule, whereas the soft endosperm was relatively free of protein bodies. The protein in the hard endosperm (white sorghum) influenced starch gelatinization. As a result, hard endosperm porridges had significantly ($P<0.05$) lower starch digestibility than soft endosperm porridges. Similar results were reported by Austin (2008). Previous studies also have confirmed that using whole grain sorghum and coarse-grain pieces in food products reduced the starch digestibility compared to using the processed grains (Austin, 2008; Palomino-Siller, 2006; Witwer, 2005).

In vivo studies showed that increased amounts of condensed tannins and total phenols significantly ($P<0.05$) lowered blood glucose level of diabetic volunteers (Thompson & Yoon, 1984). Tannin sorghums have been consumed and preferred for centuries in breads, porridges, and alcoholic beverages in Africa and Asia. Awika & Rooney (2004) stated that tannin sorghums can and should be considered as a source of natural antioxidants, dietary fiber, and colorants. Special tannin sorghums are consumed when farmers are doing field work in Africa, because these stay for longer time in the stomach, probably because of a reduced rate of digestion (Awika & Rooney, 2004). Palomino-Siller (2006) reported that the addition of 12% ground tannin bran in to bread formulation significantly ($P<0.05$) decreased starch digestibility and estimated glycemic index values. Tannins in sorghum are widely reported to reduce caloric availability and weight gain in animals. This property is useful to reduce obesity in humans and may reduce type II diabetes.

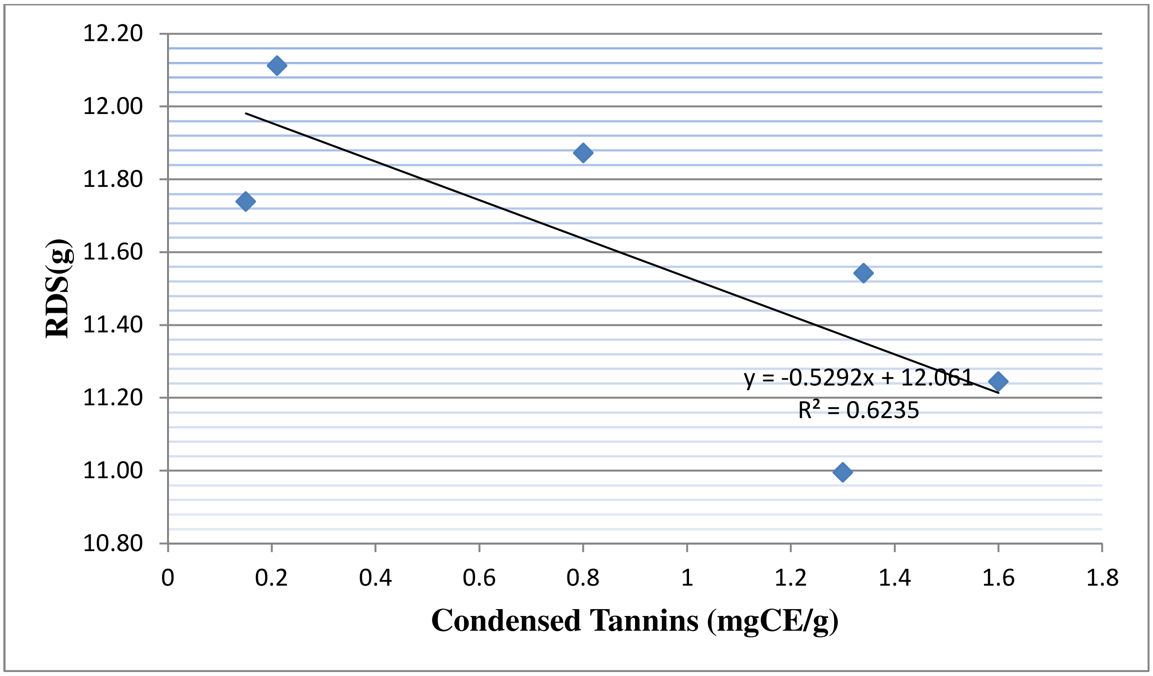


Figure 50: Correlation between *in vitro* rapidly digestible starch (RDS) and condensed tannins in sorghum extrudates

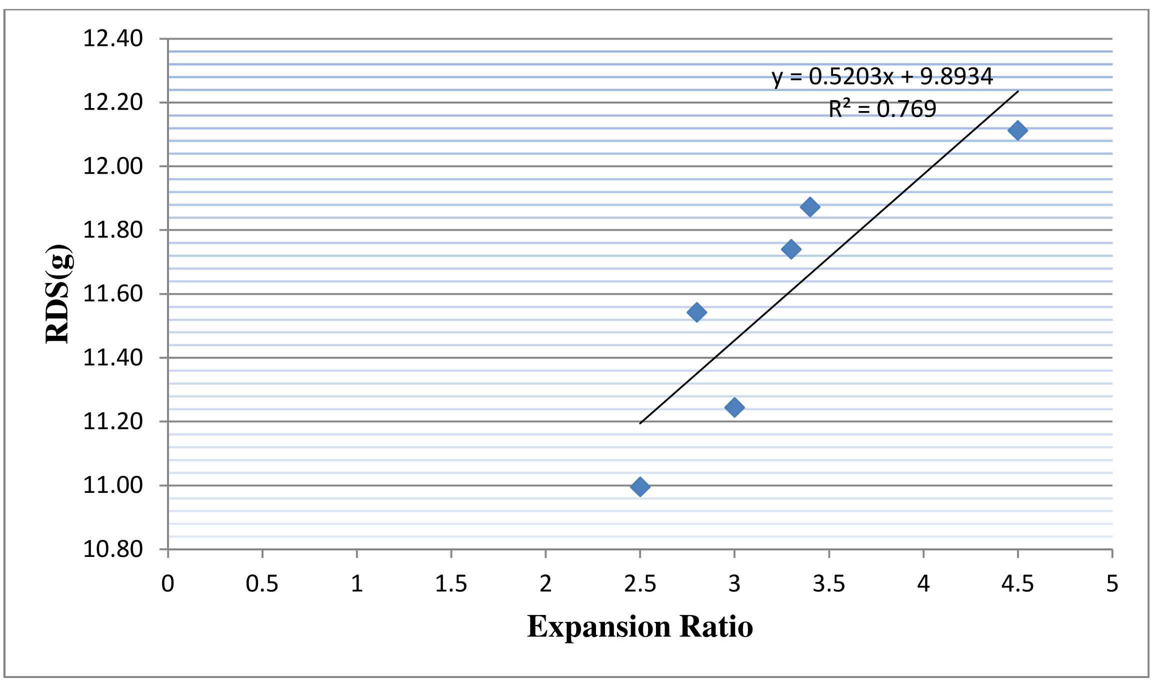


Figure 51: Correlation between *in vitro* rapidly digestible starch (RDS) and expansion ratio of extrudates

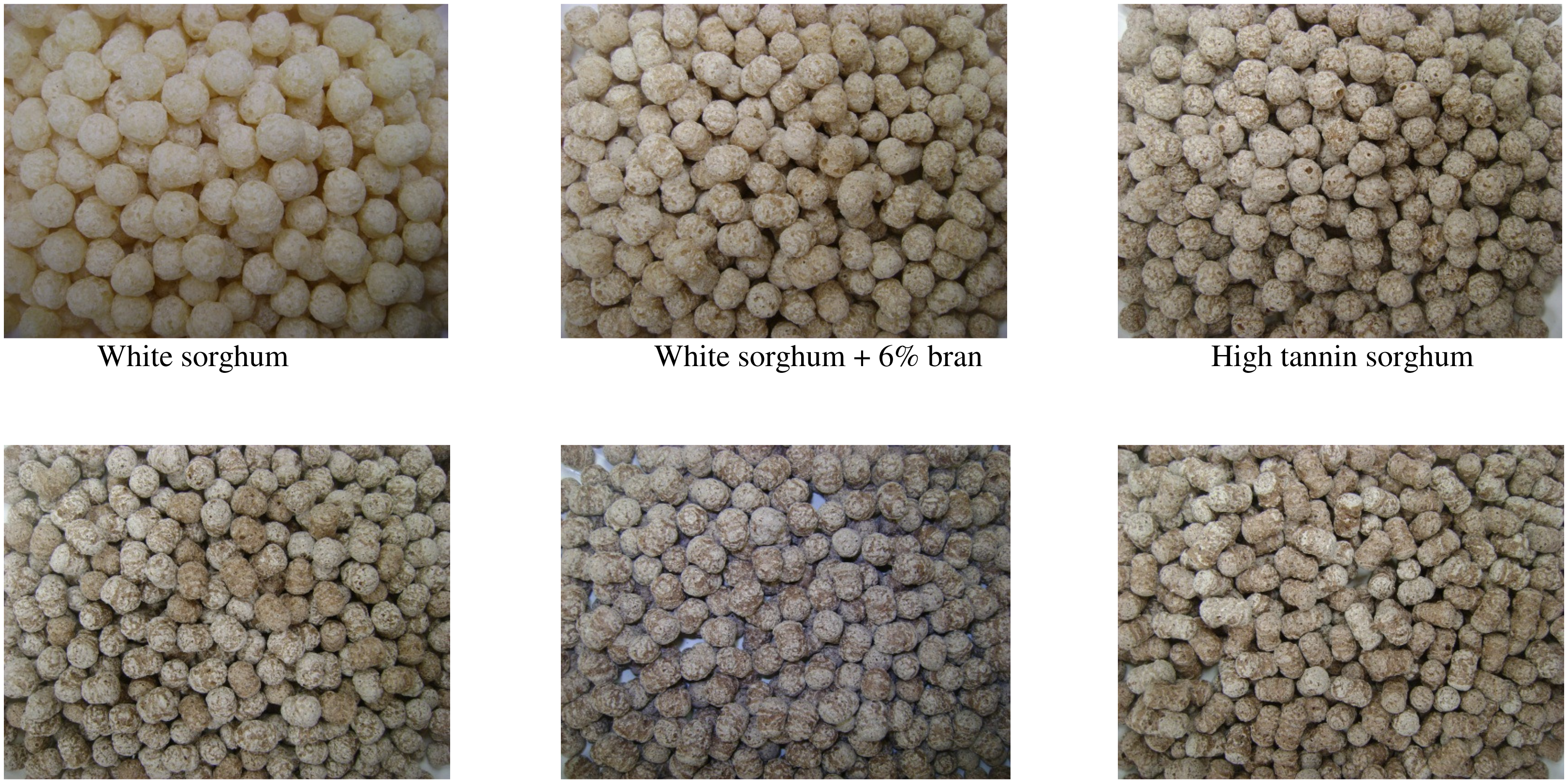
4.7.9 Sensory Evaluation of RTE Breakfast Cereals

Ready-to-eat (RTE) breakfast cereals and snacks are processed grain formulations suitable for consumption without any further cooking at home. These are favored by consumers of all ages because of their convenience, variety and high nutritional value. Most of the breakfast cereals are made primarily from corn, wheat, oat, or rice, usually with added flavor and coated with sugar and other sweeteners.

Sorghum, like other cereals, is an excellent source of starch and protein and it is a gluten-free cereal. It contains phenolic compounds which have been found to inhibit tumor development (Huang & Ferraro, 1992). High tannin sorghum bran is an excellent source of antioxidants and fiber in different food products (Rooney & Waniska, 2001).

Sorghum grain not only provides a good source of starch for expansion during extrusion but bran contains high antioxidant compounds with potential health benefits. Therefore sorghum based breakfast cereals and snacks with and without extra bran were developed by using a twin screw extruder. For sugar coating, 100g of sugar solution (65% sugar) was applied on 250g of extrudates in a coating pan.

Taste panels were 53% male and 47% female, 29% of the panelists were in 18-25 age range, 24% in 26-30, 35% in 31-55 and 12% were in 56-80 age range. This was a diverse population was 38% Caucasian, 27% Hispanic, 15% Asian, 11% African American and 9% were from other races. 60% of the panelists consumed breakfast cereal at least once a week and 15% ate breakfast cereals every day.



White sorghum | White sorghum + 6% bran | High tannin sorghum

High tannin sorghum + 6% bran | Black sorghum | Black sorghum + 6% bran

Figure 52: Sorghum based ready to eat breakfast cereals with and without additional bran after sugar coating

Table 25: Mean values of sensory evaluation of sorghum based breakfast cereals

Cereals	Appearance	Color	Taste/After Taste	Texture	Overall Acceptability	Total
White sorghum	7±1.6c	7.2±1.3c	6.7±1.4a	5.9±2.2a	6.6±1.8a	33.4
White sorghum + 6% bran	6.8±1.3bc	6.8±1.2abc	6.5±1.5a	6.9±1b	6.5±1.1a	33.5
High tannin sorghum	6.3±1.9ab	6.6±1.4ab	6±1.7a	6.7±1.4b	6.5±1.6a	32.1
High tannin + 6% bran	6.6±1.4bc	6.8±1.1abc	6.2±1.8a	7±1.2b	6.4±1.9a	33.0
Black sorghum	6.6±1.3bc	7±1.2bc	6.5±1.7a	6.7±1.2b	6.4±1.6a	33.2
black sorghum + 6% bran	5.9±1.3a	6.4±1.2a	6.4±1.4a	6.6±1.2b	6.4±1.3a	31.7

Values in each column with different letters are significantly different at $P<0.05$
n=55

Extrudates (Figure 52) developed with different types of sorghum with and without additional bran were significantly (P<0.05) different in appearance (Table 25). The taste panel liked the appearance of white sorghum extrudates without any bran because these were nicely round, but the difference was not significant from white sorghum extrudates with additional bran (Figure 53). Black sorghum based RTE breakfast cereals received the lowest ranking in appearance. This could be due to their shape which is elongated instead of round with a rough appearance. High tannin sorghum cereals stayed in middle between white and black sorghum in acceptance. Mean values for high tannin sorghum cereals with and without bran were 6.3±1.85 and 6.6±1.38 out of 9 respectively.

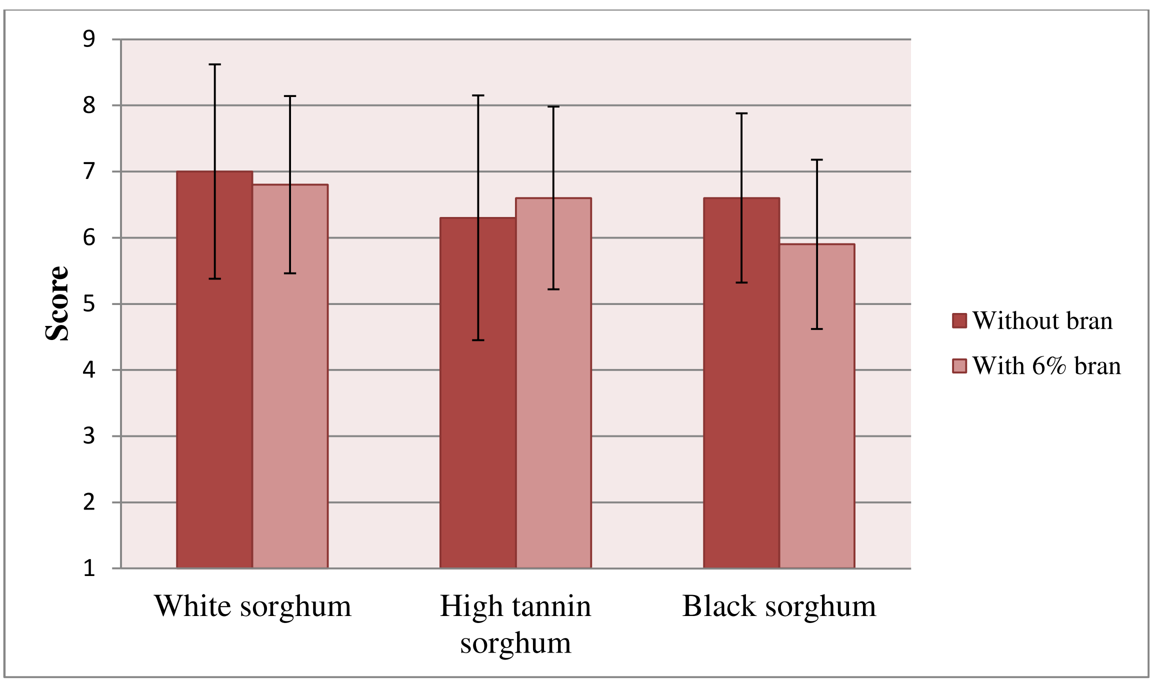


Figure 53: Appearance of sorghum based RTE breakfast cereals

All extrudates were significantly (P<0.05) different with respect to color. Panelist liked the color of white sorghum cereals most (7.2 ± 1.28) which was golden cream instead of yellow or white (Figure 52). After white, panelist liked the color of black sorghum cereals (7±1.19) (Figure 54) because of their dark brown chocolate like highly desirable color. These colors are due to presence of phenols and tannin in the pericarp of high tannin and black sorghum. Phenols in sorghums are highly associated with potential health benefits.. Consequently, fortification with black or high tannin bran will increase the phenols and improve the color of cereals too. High tannin sorghum cereals had a mean score of 6.6 ± 1.38 which was not significantly different for the color of cereals (6.8 ± 1.07) with high tannin and additional bran.

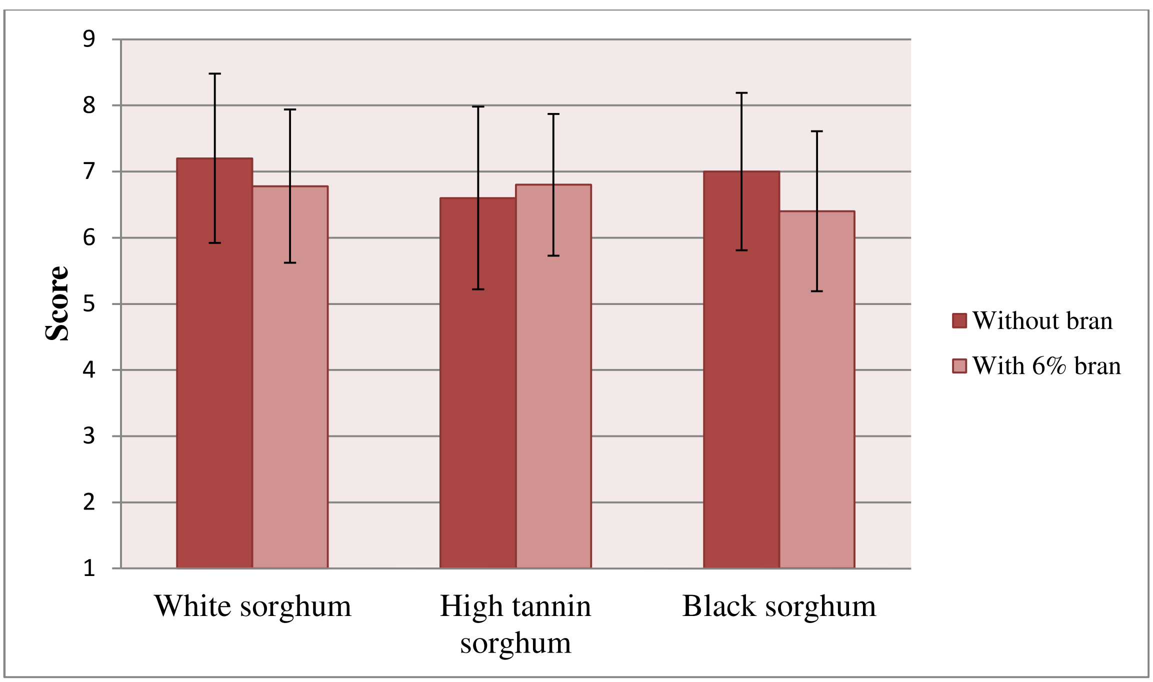


Figure 54: Color of sorghum based RTE breakfast cereals

Taste and after taste of all tested sorghum based breakfast cereals were not significantly different from each other (Figure 55, Table 25). Sensory panel ranked all the cereals between 6.01±1.71 to 6.67±1.2 out of 9. It was surprising that white sorghum cereals without bran scored highest (6.01±1.71), while white sorghum cereals with bran scored lowest (6.67±1.2), but this difference was not statistically significant. A non-significant difference in taste of all cereals could be due to post extrusion coating with sugar solution on the cereals. Sugar masked the astringent taste of tannins in high tannin sorghum extrudates. Fortification of sorghum with additional bran did not reduce consumer preference as indicated by similar ratings for extrudates from whole ground sorghum with additional bran. Acosta-Sanchez (2003) mentioned that consumers were unable to detect the flavor of white sorghum extrudates because it was bland.

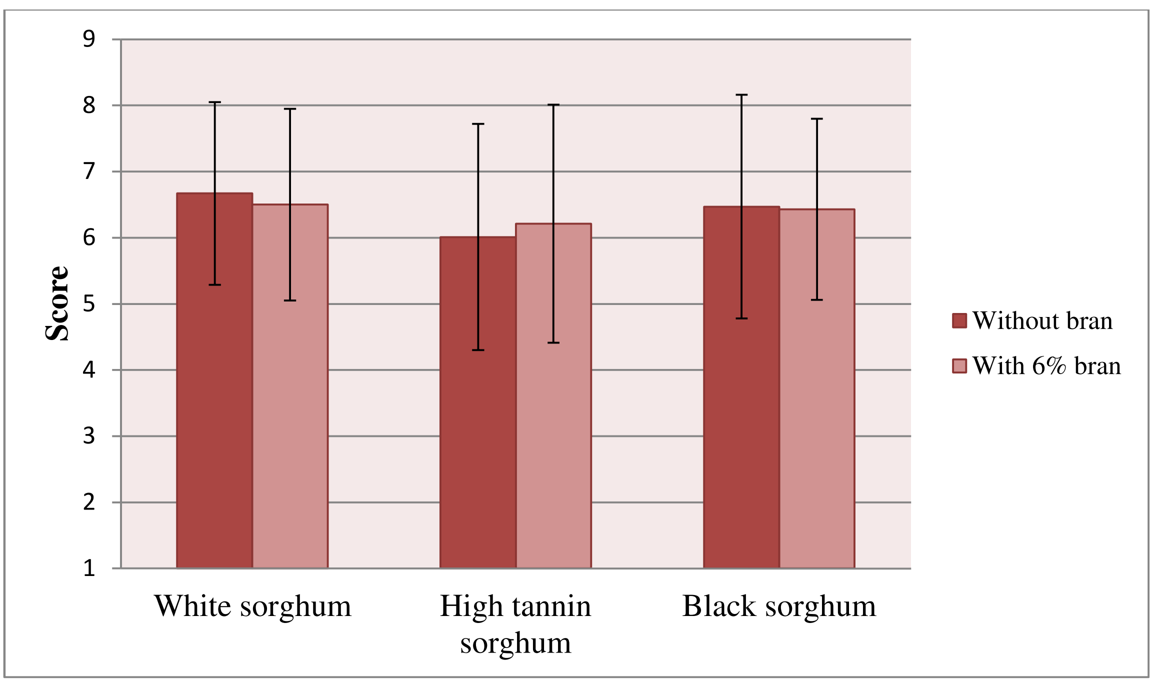


Figure 55: Taste/after taste of sorghum based RTE breakfast cereals

A significant difference ($P<0.05$) was observed in the texture of white sorghum and other cereals made with whole high tannin and black sorghum with and without additional bran (Figure 56). High tannin sorghum bran had highest (7.01 ±1.19) while white sorghum cereal had the lowest (5.96 ± 2.22) sensory panel score. The texture perceived by the taste panel was inversely correlated (R^2=0.99) to the maximum compression force (Figure 57). The consumers preferred cereals with softer texture. Thus maximum compression force (N) to break extrudates can be utilized to predict consumer perception of texture. These results agree with the findings of Acosta-Sanchez (2003). Perez-Gonzalez (2005) mentioned that crunchy texture and gritty appearance of the whole grain sorghum extrudates were liked by consumer sensory panel.

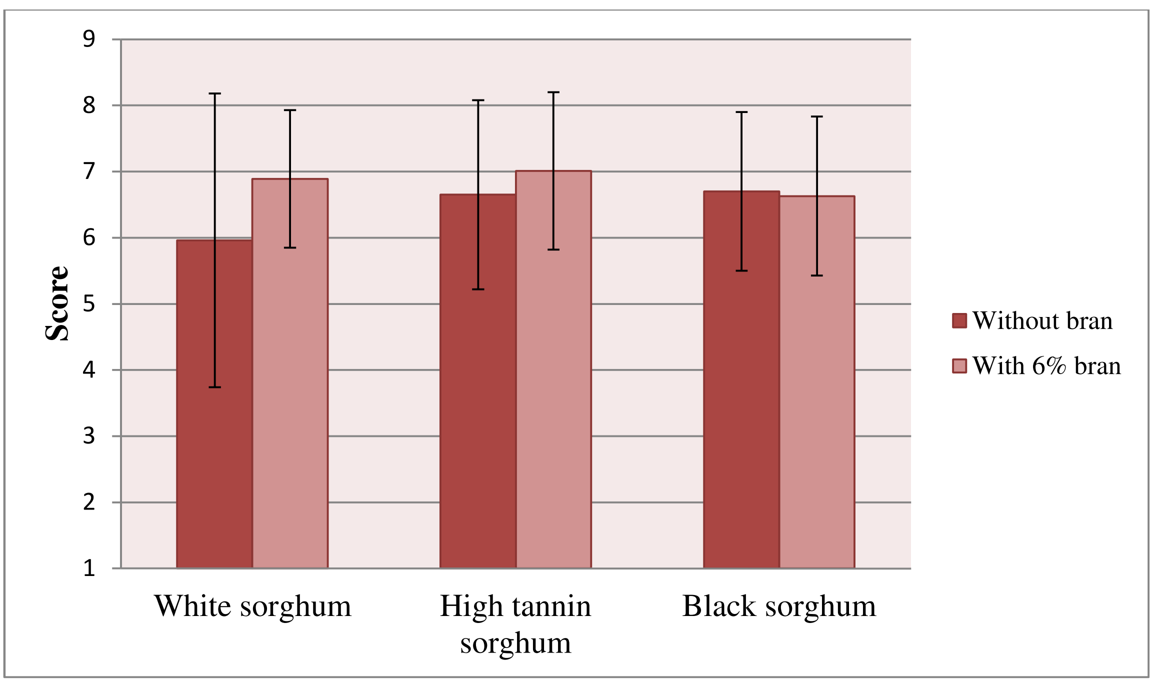


Figure 56: Texture of sorghum based RTE breakfast cereals

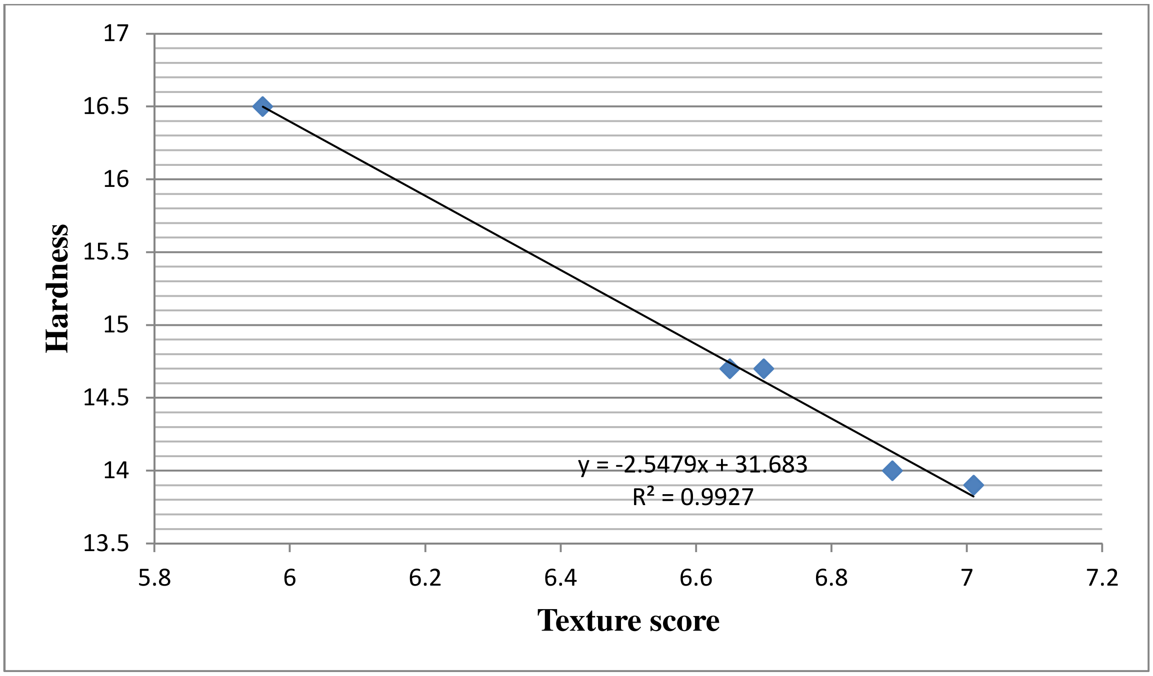


Figure 57: A correlation between texture score of sensory evaluation and hardness (maximum compression force)

There was no significant difference in the overall acceptability (Figure 58) of different types of sorghum based breakfast cereals with and without additional bran. Sensory panel did not have a significant preference. The overall liking score received by all types of cereals ranged from 6.4–6.6 out of 9, which is about 71-73% of total score respectively. Therefore, consumers liked the cereals made with white, black and high tannin sorghum equally. High tannin sorghum with 6% bran breakfast cereals were liked for their texture, however for taste and the after taste, sensory panel preferred the white sorghum cereals without additional bran. White and black sorghum cereals were considered the best in color with no significant difference except appearance white sorghum was ranked significantly higher than that of black sorghum. Whole grain sorghum flour is a wholesome, hearty grain that provides fiber with a mild flavor that does not compete with the delicate flavors of other food ingredients (Rees & Henneman, 2009). Lu (1986) developed flakes by using whole and decorticated sorghum, sensory results indicated that both types of sorghum flakes were palatable and acceptable to people. During sensory evaluation, some panelists commented about attributes of sorghum based cereals (Table 26).

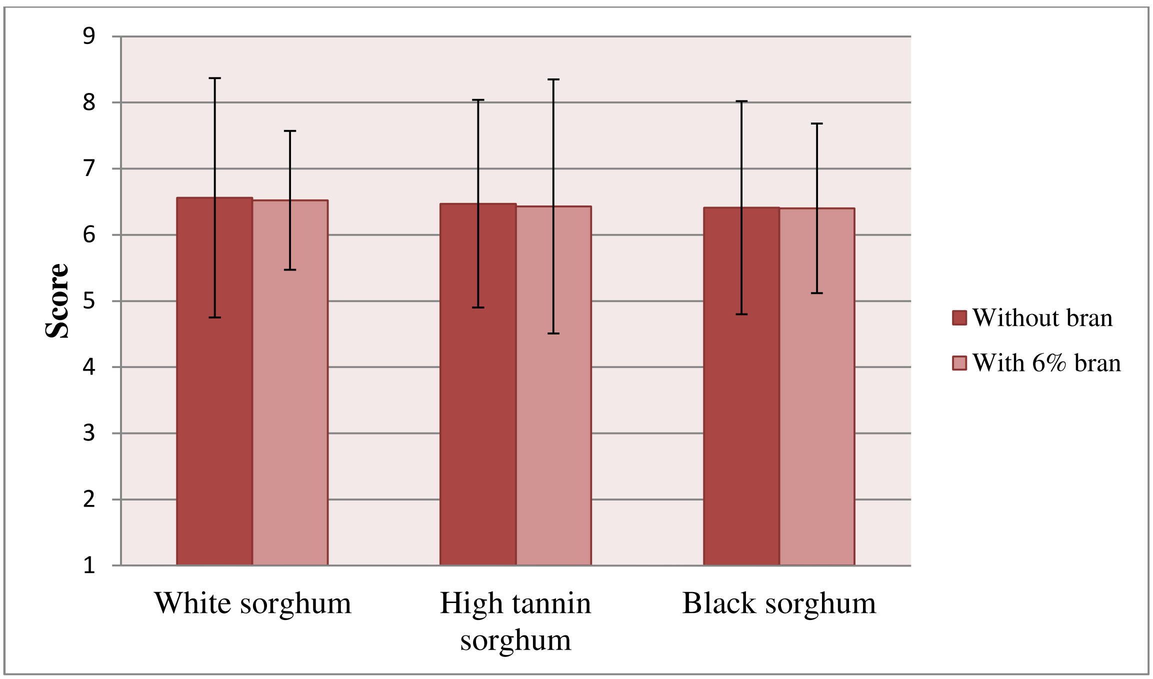


Figure 58: Overall acceptability of sorghum based RTE breakfast cereals

Table 26: Comments of consumer sensory panel about sorghum cereals

Type of cereal	Comments
White Sorghum	Very porous, easy to destroy, no aftertaste, too big but good ball shape, perfect, too easy to eat, crunchier texture, dissolve quickly in mouth, stable in milk, taste a bit bland in milk but very good.
White Sorghum+ 6% Tannin Bran	No after taste, not round enough, too soft in milk, bland flavor, stable in milk, stronger taste without milk, texture became soggy with milk
High Tannin Sorghum	Very porous on surface, viscous when added to milk, other taste not strong, good ball shape, perfect, color is masked by sugar coating, texture is better, stick to teeth, slight off-flavor, granular mouth feel was positive, texture stays crunchy in milk
High Tannin + 6% Tannin Bran	A bit strong after taste, viscous when added in milk, perfect round shape, best taste in milk, slightly granular but not bad, dissolve in mouth but stable in milk, taste and texture better without milk,
Black Sorghum	After taste but barely detectable, too long, very good taste, kind of disappear in mouth, flavor and texture is better without milk,
Black Sorghum + 6% Tannin Bran	Not look like a ball, after taste is not strong, too long, rough appearance, shape is not good, dissolve in mouth but stable in milk, taste was stronger without milk but with milk it was ok,

5. SUMMARY

Sorghum is used in ready to eat breakfast cereals and snacks due to its bland flavor, low cost, high antioxidant and fiber contents and with processing almost similar to corn flour. It was used in gluten containing and gluten free breakfast cereals and snacks in four experiments. In the first experiment, gluten containing cereals and snacks were developed by using 0-40% high tannin whole ground sorghum and 0-10% high tannin bran. By using 40% sorghum with 10% additional high tannin bran, the bulk density of cereals was increased about 77% and expansion ratio of extrudates was decreased about 25%, however cereals obtained were 20% darker (L*) than that of control. In this experiment different processing conditions were used. By adjusting the levels of bran in the formulation and extrusion parameters, a product with good quality characteristics could be developed.

In the second experiment gluten free breakfast cereals were developed by using 50 and 60% whole ground white sorghum and 5-10% high tannin sorghum bran. Expansion ratio was decreased for 60% sorghum and 10% bran, and as expected bulk density was increased. Bowl life was increased to 18 min with 60% sorghum and 10% bran. There was no significant difference in hardness of both types of extrudates but WAI for 60% sorghum and 10% bran was significantly less than that of 50% sorghum and 5% bran containing extrudates. This indicated that extrudates contained 60% sorghum and 10% tannin bran were less porous and contained a stronger network of starch and bran particles. This ultimately reduced its WAI and increased bowl life. Antioxidant activity was significantly different in both extrudates and varied from 20-49 μmolTE/g. In this experiment we found that whole ground white sorghum and high tannin bran in combination with other gluten free ingredients could be used to develop RTE breakfast cereals. These gluten free cereals have long bowl life, optimum bulk density and expansion ratio, high fiber and antioxidant activity with creamy white color.

In the third experiment, 80% white, high tannin and black sorghum were used without any additional bran to develop gluten free breakfast cereals and snacks purpose of this experiment was to see how far we can go by using whole ground sorghum instead of corn flour. This time, the extrudates were more expanded with reduced bulk density. But this increased expansion had adverse effect on bowl life. As a result bowl life of cereals was reduced (3 min) compared to 50 and 60% white sorghum and 5-10% bran containing extrudates. Thus, increased bran contents increased the hardness of extrudates and decreased the porosity (Yanniotis et al., 2007) which helped to increase bowl life. Bulk density of extrudates in experiment 3 was lower than that of extrudates developed in previous experiment while expansion ratio was higher.

Extrusion may have a detrimental effect on the antioxidant properties of sorghum extrudates. Antioxidant activity retained after extrusion by the extrudates having 80% of high tannin and black sorghum extrudates was 73 and 85%, respectively. For white sorghum extrudates, the antioxidant activity was increased by 144% because of solublization of phenolics present in the cell wall of sorghum which made them available for analysis. Extrudates developed in experiment 3 were low in bulk density and bowl life, but were highly expanded and had significant retention of antioxidant activity. Such characteristics made these extrudates a preferred savory snack with cheese seasoning instead of breakfast cereals. Thus whole ground white sorghum is a feasible option for snacks because of its versatility, product characteristics, cost and processing properties.

In experiment four, gluten free RTE breakfast cereals and snacks were developed by using 85% whole ground white, high tannin and black sorghum with additional high tannin bran. Objective of this experiment was to add additional bran to increase the bowl life, increase bulk density and antioxidant activity and fiber contents. In these extrudates by increasing bran level, bulk density was increased while expansion ratio and L*values were decreased. Overall the expansion of whole ground black sorghum extrudates was quite different compared to other extrudates because of the hard pericarp. Hardness of extrudates decreased with increasing bran level except the hardness of whole ground black sorghum extrudates. There was more force required for crushing black sorghum extrudates with additional 6% bran. This was probably because of dense cell walls of these extrudates. Austin (2008) found that black sorghum bran was hard when compared to white and high tannin sorghums.

Bowl life of extrudates in experiment four, varied significantly between 5.3 to 14 min. Bowl life was low for the extrudates without additional bran and increased as additional bran was added. Bowl life was 8.7 min for the white sorghum extrudates and 14 min for high tannin sorghum with 6% additional bran. A positive correlation between water soluble index and expansion ratio (R^2=0.89) indicated that the more expansion ratio provided a large surface area for water to interact with starch and other soluble components (Lee at al., 1999). In other words, more expanded cereals had less bowl life. The WSI was significantly higher for the extrudates without additional bran and decreased as additional bran was added. The WAI depends upon the availability of hydrophilic groups which bind water molecules and on the gel formation capability of macromolecules. The WAI of extrudates ranged from 2.5 to 5.9 g/g, as particle size of feed was increased, WAI value decreased. Hashimoto and Grossmann (2003) reported that by increasing fiber level and by decreasing starch contents, the WAI of extrudates increased. However an increase in bran contents tended to decrease the WSI. These characteristics of 85% sorghum and 0-6% high tannin bran containing extrudates made them very good RTE breakfast cereals and snacks. These cereals have reasonable bulk density, better expansion ratio, high fiber contents and optimum bowl life with attractive colors varying from creamy white to chocolate brown.

Whole ground sorghum grains had 0.9-12.6 µgGAE/g total phenols, 13.3-193.3 µmolTE/g antioxidant activity and 0-8.9 mgCE/g of condensed tannins. High tannin sorghum bran had 33 µgGAE/g total phenols, 368.7 µmolTE/g antioxidant activity and 23.2 mgCE/g condensed tannins. Extrusion cooking significantly reduced the measurable total phenols for all three types of sorghum. The retention of total phenols varied from 13-41%. Extrudates with additional high tannin sorghum bran had more total phenols than extrudates without it.

Whole ground white, high tannin and black sorghum composite flours with and without additional high tannin bran had significant reductions in antioxidant activity after extrusion. The extruded samples retained 21% and 40% of antioxidant activity for high tannin and black sorghum without additional high tannin bran respectively. The retention was increased up to 30% in high tannin sorghum extrudates when additional

6% high tannin sorghum bran was added. The significant reduction in antioxidant activity in cooked high tannin sorghum can be attributed largely to the interaction of tannins with prolamins (Emmambux & Taylor, 2003) which made them unavailable for antioxidant assay. Effect of extrusion on the condensed tannins was detrimental. Their retention was varied from 12-28%. Similarly Ngwenya (2007) found that extrusion cooking reduced measurable tannins by almost 97% in whole grain extrudates when she used a twin screw extruder. The smaller particles promoted interactions during extrusion, lowering condensed tannins and antioxidant activity.

Phenolic compounds in the pericarp and testa of high tannin and black sorghum varieties reduced the digestibility of starch. All sorghum based extrudates had significantly ($P<0.05$) lower starch digestibility than that of corn flour extrudates. All types of sorghum had non-significant difference in starch digestibility from 0.5-2hrs. After 16 hrs high tannin sorghum containing extrudates had the lowest starch digestibility (79%) which was significantly different from other sorghums. There was a negative correlation between the RDS and tannin contents (R^2=0.62). Increasing the tannin contents in the extrudates decreased the digestibility of starch. But white sorghum did not contain tannins, therefore incomplete starch gelatinization could also slow starch digestibility. A positive correlation between RDS and expansion ration (R^2=0.77) indicated that the most expanded extrudates were more rapidly digested by α–amylase. Previous studies also confirmed that using whole grain sorghum reduced starch digestibility (Austin, 2008; Palomino-Siller, 2006; Witwer, 2005). Special tannin sorghums are consumed in Africa when farmers are doing field work, because they stay for longer time in the stomach, probably because of a reduced rate of digestion (Awika & Rooney, 2004). My results indicated that high tannin sorghum reduced the starch digestibility which benefits type II diabetics.

The appearance, color, taste, texture and overall acceptability were evaluated using a hedonic scale from 1 to 9. Breakfast cereals made from different types of sorghum and bran levels were statistically equally rated in taste and overall acceptability. The taste panel gave lower scores for appearance and color to extrudates made from whole ground black sorghum with additional bran compared to those produced from white and high tannin sorghum. Cereals made from high tannin sorghum and additional bran had the best texture because additional bran increased the bulk density and made it crisp. On the other hand panel did not like the texture of white sorghum because it was very soft. The texture perceived by the taste panel was inversely correlated (R^2=0.99) to the maximum compression force thus consumers preferred cereals with softer texture.

White and high tannin sorghum with and without additional bran can be used to develop different types of breakfast cereals and snacks because of their versatility and cost. The desired quality characteristic of final products, gluten free or gluten containing requirements, and other nutritional parameters will decide how much whole ground sorghum with or without additional bran would be used. This will also necessitate the processing condition of extruder, therefore by changing the moisture contents and extrusion parameters, desired products can be developed. The color and taste of the extrudates from white sorghum are white and bland which promote their use in a wide variety of simple snacks that can be baked or fried and flavored. Extrudates of high tannin sorghum have a strong, dark brown, highly desirable color. Specialty sorghum like high tannin and black can be used to develop the naturally colored breakfast cereals and snack. It can also be used to reduce the quantity of cocoa in cocoa puff type breakfast cereals.

We also found that in all the four experiments, the maximum retention of antioxidant activity was in experiment 3. In this experiment we used very harsh extrusion conditions as compared to other three experiments. It indicated that by using harsh extrusion conditions either by using twin screw or friction type dry extruder retention of antioxidant activity in high tannin and black sorghum extrudates can be increased. In these experiments, twin screw extruder was used but friction-type, short-barrel, single-screw extruders can also be used to produce snacks similar to corn curls in different shapes and sizes. The acquisition of sorghum for processing can be challenging but it is becoming more available because of its positive properties. Some suppliers of sorghum in USA are listed in Appendix C.

Suggested further works should include:

- Whole sorghum and bran should be milled more efficiently for finer particles size distribution.
- Different fractions of milled whole sorghum should be used to develop breakfast cereals and should be analyzed to see the effect of particle size on antioxidant activity of extrudates.
- Black sorghum bran should be used in breakfast cereals to confirm the effect of its hardness on the texture and shape of extrudates.

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APPENDIX A

Work sheet for preparing and presenting the sample in consumer test

Date: 01-12-10 **Work Sheet for consumer testing**	
This sheet will be posted in the area where samples are prepared and coded	
Types of sample: Soft drinks	Type of test: Consumer test
Sample identification	Code
311	A (587, 211, 894)
314	B (214, 413, 457)
315	C (751, 215, 365)
318	D (246, 730, 218)
319	E (319, 915, 511)
322	F (777, 322, 433)
Serving cups should be coded as below Panelist #	Order of Presentation (1-6) and so on
351, 216, 522,138, 496, 984, 869, 375, 743, 274	ABCDEF
797, 967,665, 635, 593, 854, 711, 448, 129, 966	BCAFED
289, 532, 287, 372, 113, 581, 228, 445, 976, 862	EFDCBA
145, 859. 657, 334, 799, 917, 355, 824, 793, 688	FACBDE
463, 728, 572, 246, 139, 461, 365, 542, 881, 734	EFBACD
874, 191, 959, 698, 423, 117, 276, 332, 691, 549	DCABEF
Sticker will placed on the serving cups Samples will presented from left to right on the tray Codes will also written on the sheets of panelists	

APPENDIX B

Panelist #_______

Sensory Evaluation Performa

Please complete the information below:

Age:

☐ 18-25	☐ 26-30	☐ 31-35	☐ 36-40
☐ 41-45	☐ 46-50	☐ 51-55	☐ 56-60
☐ 61-70	☐ 71-80	☐ Over 80	

Gender:

☐ Male ☐ Female

Ethnicity

☐ Caucasian
☐ African American/Black
☐ Hispanic
☐ Asian
☐ Native American
☐Other

About how often do you eat breakfast Cereals?

☐ Everyday
☐ At least once a week
☐ Once every two weeks
☐ Once a month
☐ Never

Do you suffer from any food allergies?

☐ Yes
☐ No

Instructions:

- You will be testing six samples of breakfast cereals with 2% milk.
- Make sure to use the ballot with the sample number that matches the number of the sample.
- Eat Ricotta cheese and cracker and drink water to rinse your mouth before you evaluate each sample and as needed throughout testing.

SAMPLE: _______________

Please check one box that represents your response

How much do you like or dislike the APPEARANCE of sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How much do you like or dislike the COLOR of sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How much do you like or dislike the TASTE and AFTER TASTE of sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How much do you like or dislike the TEXTURE of sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate your OVERALL ACCEPTABILITY of sample

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Additional Comments:

___

Sensory Evaluation in Photos

APPENDIX C

Suppliers of Sorghum in USA

ADM Milling

8000 W. 110th Street

Overland Park, KS 66210

AgVantage Natural Enterprises

109 First Street

New Cambria, KS 67470

Bob's Red Mill

13521 SE Pheasent Court

Milwaukie, Oregon 97222

Twin Valley Mills LLC

PRI Box 45

Ruskin, Nebraska 68974

VITA

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